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TEXT-BOOKS OF ANIMAL BIOLOGY

Edited by JULIAN S. HUXLEY, M.A.

Honorary Lecturer in Experimental Zoology, King's College, London

THE DEVELOPMENT OF SEX IN VERTEBRATES

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THE DEVELOPMENT OF SEX IN VERTEBRATES

BY

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WITH A PREFACE BY

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AUTHOR'S PREFACE

THIS book is concerned with one of the most fruitful fields of recent research : the embryology, physiology, and histology of the gonads. Moreover, it is certain that further research will yield to those who devote themselves to the subject an even richer harvest in the immediate future. The primary object of the book, therefore, is to interest those who are about to enter on a biological career.

The author has attempted to treat the subject as it interests him, believing this to be the most likely way to interest others This must be his excuse for the omission of reference to much of the earlier work on the subject, and the treatment of the whole from a somewhat unorthodox aspect.

The book is intended as a contribution towards the correlation of the morphology and physiology of the gonads, and the genetics of sex-determination in vertebrates. The antithesis between morphology and physiology, which has been long apparent, has been emphasised in modern times. The study of structure has become further and further removed from that of function and new subjects have arisen to meet the requirements of increased specialisation. Nowhere has this divergence been more apparent than in the study of reproduction in vertebrates. Anatomy, physiology, and genetics have split up between them this single field, and the knowledge concerning each branch has accumulated more or less independently of the others. Yet the subject is one, and the correlation of the results obtained in each branch is both necessary and urgent. Moreover, this synthesis should result in a fuller understanding of the problems involved and should be mutually beneficial to those working on the various branches concerned by enabling them to view the subject as a whole.

The mechanism which determines sex and the evolution

of the structures and functions of the gonads in vertebrates are discussed. These two problems, the determination of sex and the evolution of the gonads, are quite distinct, but the evidence bearing on them is interwoven. The one belongs to ontogeny and the other to phylogeny. Some of the more general conclusions concerning these two major problems which may be drawn from the material presented are summarised in the concluding chapter. The scope of the book has been limited to vertebrates because most of the recent advances in our knowledge of sex have applied to them. It did not seem advisable to complicate the issues by dealing with invertebrates (see Goldschmidt, *The Mechanism and Physiology of Sex Determination*), except in a few instances in which our knowledge of them is relevant to problems presented by vertebrates. Moreover, in order to limit the issues still further, no attempt has been made to deal systematically with the accessory sexual organs or secondary sexual characters (see Crew, *The Genetics of Sexuality in Animals*, and Lipschütz, *The Internal Secretions of the Sex Glands*).

The author wishes to take this opportunity of expressing his indebtedness to Professor J. P. Hill, F.R.S., under whose direction most of the researches, referred to in this book, for which the author is partly or wholly responsible were performed. The author's thanks are due to his wife for much help and encouragement; to his friend and collaborator Dr. A. S. Parkes for his advice, criticism, and many valuable suggestions; to Mlle. K. Ponse for courteously communicating to him the results of her researches on toads, some of which are still unpublished; to Professors J. Brontë Gatenby, J. S. Huxley, and D. L. Mackinnon for reading and criticising the manuscript; to Mr. R. F. Adgie for reading the proofs; and to Professor D. M. Blair, Dr. A. S. Parkes, Miss R. Deanesly, and Mr. C. C. Hentschel for the loan of material from which Plate I, Fig. 2, Plate XV, Fig. 3, and Fig. 1 and Text-fig. 23 (*a*) respectively were prepared. He is indebted also to the authors referred to in the legends who granted him permission to reproduce figures, and especially to Professor

E. B. Poulton, F.R.S., Professor R. Goldschmidt, Professor E. Witschi, Dr. C. J. Bond, C.M.G., and Dr. R. R. Humphrey for the loan of the originals of their figures, and to the Council of the Royal Society for Pls. III, VI, IX to XIV, XXII, Text-figs. 11, 24, and 25; the Council of the Royal Irish Academy for Pl. XVII, Fig. 1; the Council of the Entomological Society of London for Pl. XXIV, Fig. 2; the Council of the Zoological Society of London for Pl. I, Fig. 1, Pl. XVI, Text-figs. 10 and 13; L'Association des Anatomistes for Text-fig. 7; La Société de Biologie for Text-fig. 16; the K. Vetenskapsakademiens, Stockholm, for Pl. IV; the Wistar Institute of Anatomy and Biology for Text-figs. 4, 6, and Pl. XX; the Clarendon Press, Oxford, for Text-figs. 2 and 3, Pls. II and III; the University Press, Cambridge, for Pls. XV, Fig. 2, Pl. XXIII, Text-figs. 17 and 21; Herrn J. F. Bergmann for Text-fig. 8; Messrs. Sherratt and Hughes for Pl. XVII, Fig. 2; Herrn Julius Springer for Pl. XXI, Figs. 1 and 2; Herrn Georg Thieme for Pl. XXIV, Fig. 1; l'Archive de Biologie for Pls. V and VII, Text-fig. 9; the Quarterly Journal of Experimental Physiology, for Text-fig. 12; the Science Press for Text-fig. 5. Messrs. F. C. Pittock, D. A. Kempson, A. V. Cobbett, and R. S. Cockrill rendered much valuable assistance in the preparation of the illustrations.

F. W. R. B.

King's College,
London.

April, 1930.

EDITOR'S PREFACE

If the aim of General Biology be to link up the results of separate sub-sciences and fields of research, then it is, I think, fair to say that the general biology of sex is more advanced than that of any other topic.

The inheritance of sex itself, of sex-linked and of sex-limited characters, has provided interesting special cases of Mendelian law, often beautifully mirrored in the visible cytological facts. One of the fundamental principles of neo-Mendelism, the idea of genic balance, was first worked out in regard to sex-determination. Meanwhile on the physiological side the complete dependence of many secondary characters upon the hormones of the gonads was demonstrated ; the gonad and the thyroid have to their credit the most striking and clear-cut cases of morphogenetic change produced by hormones. At first the work on the gonads fell short of that on the thyroid in that efficient gonad extracts were not known. But now, with the preparation first of ovarian and, quite lately, of testicular extracts capable of exercising a positive effect on the sexual characters of animals of other species, families or even classes, this gap has been filled, and a new and exciting chapter of chemical biology lies open.

And just as the theory of genic balance was first proved in the field of sex-determination, so in that of the control of secondary sex-characters the interaction of hormones has been diagrammatically demonstrated. The interlocking of pituitary and ovary is clear and definite ; that of pituitary and testis is definite though rather more obscure ; that of gonad and thyroid, as revealed by the study of hen-feathering in fowls, promises to be of equal interest.

In quite another field, the discovery that hormonic control was lacking in insects reminded us that groups of animals can be as different in their physiology as in their morphology; while the gynandromorph birds, so clearly discussed by Dr. Brambell in one of the chapters of this book, provide a link between the insectan and the vertebrate conditions, by showing that in the tissues of vertebrates, hormone control appears to be superimposed upon a chromosome control.

Meanwhile in still other fields the methods of modern genetics were casting light upon the biological value of sex and demonstrating exactly how it promoted evolutionary variability; while work upon conjugation in Protozoa demonstrated the converse—that the rejuvenating effect of conjugation was not due to the sexual exchange at all, but to the concomitant process by which a senescent macronucleus is replaced from the germinal micronuclear reserve.

In still another domain three main lines of work combined to throw light on the developmental physiology of sex: the study of human and lower vertebrate hermaphrodites, intersexes, and other sexual abnormalities; the study of sex-reversal, notably in Amphibia, later in fish, fowls, and other creatures; and finally the study of the intersexes produced by race- and species-crosses. This last line of work, in the able hands of Goldschmidt, linked up the physiology of sex-development with the Mendelian basis of sex-determination; and his ideas, first worked out for moths, have been fruitfully applied to mammals, birds, and amphibians. The fundamental notion, first developed by Goldschmidt for sex-genes, that their varying potencies depend primarily upon differences in their rate of action during ontogeny, seems likely to be as universal in its application to genes in general as is the theory of genic balance, first studied in relation to sex-determination, for the determination of all characters.

Finally, the studies of various physiologists, of Riddle on pigeons, of Manoilov, Blakeslee and others on chemical sex-tests, are correlating sex-differences with differences in metabolic rate.

Dr. Brambell's book aims chiefly at filling one important gap in this broad subject of sex. It aims at giving, in the light of our modern conceptions of the biology of sex, a description and analysis of the steps by which, in vertebrates, the sexual characters are gradually realised as the final result of the initial chromosomal sex-determination ; and he pays special attention to one field hitherto somewhat neglected in general works on sex, namely, the development and histology of the main link between chromosomes and characters—the gonads themselves.

Throughout, the modern biological point of view (which is also the old-fashioned one revived) is stressed—the close interrelation of structure and function. Though the comparative and evolutionary motive runs all through the book, no one can accuse the author, as Radl, in his *History of Biological Theories*, accused the bulk of late nineteenth-century morphologists, of spending their time " comparing one thing with another without taking the trouble to find out what either of them really is."

Both in this respect and in summarising new research (much of it the work of Dr. Brambell and his collaborators) that has not yet found its way into the text-books, I believe that this book will prove of considerable value. It both usefully sums up existing knowledge, and points to the places where new knowledge and new research are needed. It should be of service both to research workers and to students interested in the general biology of sex.

Julian S. Huxley.

King's College,
London.
March, 1930.

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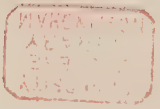
THE DEVELOPMENT OF SEX IN VERTEBRATES

CHAPTER I

REPRODUCTION IN VERTEBRATES



THIS book is concerned with the mechanism by which the sex of an individual is determined and with the embryonic development and evolutionary history of the reproductive systems of vertebrate animals. The fundamental problems of the nature of sex and the biological significance of sexual reproduction are not discussed, as they are outside this province.

Sex consists essentially in the power to produce gametes, either spermatozoa or ova; all other sexual functions are subsidiary to this. An animal is therefore a male if it has a testis and a female if it has an ovary. These organs, known collectively as the gonads, constitute the *primary sexual organs*. The structures concerned with the liberation of the gametes, or ova and spermatozoa, and with copulation and gestation, such as the oviducts, uteri, vasa deferentia, penis, vagina, etc., constitute the *accessory sexual organs*. There are, in addition, certain characters associated with sex, such as the plumage of birds, the long hair of women, the antlers of deer, etc., which are not directly concerned with reproduction. These characters are known as the *secondary sexual characters*. The development of both the accessory organs and the secondary sexual characters in vertebrates depends very largely on the presence of either an ovary or a testis, and, to this extent, only indirectly on the causes which determined the sex of the gonads. There are, therefore, two distinct problems concerning the sexual

development of an individual: the nature of (*a*) the factors determining the sex of the gonads, and of (*b*) the factors determining the sex of the accessory sexual organs and the secondary sexual characters. The former is the problem of sex-determination *sensu stricto*. The latter concerns the influence of the ovary and the testis on development. The confusion that has arisen concerning the development of sex in vertebrates is due to a large extent to a failure to distinguish between these two issues. The gonads appear to have little or no influence upon the development of the accessory organs and secondary sexual characters in Insects and possibly other invertebrates, where the development of the whole reproductive system apparently depends on the initial factors which determine sex.

A comparative survey of the structure of the reproductive organs in the various vertebrate groups will afford a useful introduction to the subject of this book and will provide a basis for a more detailed conception of their function and evolution.

Primitive Segmental Arrangement.—There can be no doubt that the primitive arrangement of the gonads in vertebrates is segmental. Such an arrangement is found in the Cephalochordates. The gonads in Amphioxus, whether ovaries or testes, are arranged in two rows along the sides of the body and form two ventro-lateral ridges in the region of the atrium. The number varies in different species and in different individuals, but Amphioxus generally has twenty-six pairs. The curious rudimentary ovary, known as

FIG. 1.—Lateral view of Amphioxus, showing the segmentally arranged gonads in black.

Bidder's organ, which is found attached to the anterior end of the testis or the functional ovary in the toad may have also a segmental origin. Ponse [194] has adopted the suggestion of Swingle that it represents a progonad (Chapter VII). The

testis in the Gymnophiona consists of a row of rounded bodies connected by a common collecting duct. This arrangement is probably also of segmental origin, although the ovaries, which are in the form of a pair of narrow bands, do not exhibit it.

The gonads of all other adult vertebrates consist of a single pair, although, as will be shown later, indications of a segmental origin can be seen in the development of several forms.

Cœlomic Origin.—The gonads are developed in the wall of the cœlom, into which they freely project. They are covered on their free surfaces by the cœlomic peritoneum which forms the germinal epithelium. This covering is retained throughout life in the female, but disappears from the outside of the testis during development in the male. The ovaries and testes of many teleost fish have become converted into hollow sacs which are continuous with the ducts that lead from them to the exterior. This arrangement is secondary and has resulted from the growth and ultimate fusion of the lateral margins of each gonad. In consequence the cavity of the teleost gonad is morphologically of cœlomic origin and the germinal epithelium which lines it has been derived from the peritoneum.

The ovaries of many Sauropsida and Mammalia are almost entirely enveloped by the funnel of the oviduct, and, in some mammals, a complete ovarian capsule is formed by this funnel and by the upgrowth of ridges of the body wall. Another modification is exhibited by the males of higher mammals in which the testes descend into scrotal sacs which are more or less completely separated from the body-cavity. The cavity which surrounds the gonads in all these modifications is, however, morphologically a part of the cœlom.

Liberation of Germ-Cells.—The primitive method of escape of the germ-cells in vertebrates was by perforation of the wall of the gonad and liberation into the cœlom. In Amphioxus and its allies the germ-cells then burst through the body wall into the atrial cavity and thus escape from the narrow cœlomic sac which surrounds, but is more or less filled by, the mature gonad. In the Cyclostomes the germ-cells escape in a similar manner into the body cavity from which

they find their way to the exterior through the genital pores. This primitive method of liberation of the germ-cells into the cœlom is retained in the females of all vertebrates. The ova are taken up from the body cavity by the oviduct and are ultimately conveyed to the exterior. The males of the higher vertebrates are much modified in this respect and the spermatozoa do not escape through the walls of the testis into the body cavity. Indeed, such a path is rendered impossible by the development of a thick sheath of connective tissue, the tunica albuginea. Instead, the spermatozoa are conveyed from the spermatic tubules to the efferent duct through a network of fine tubules.

The Testis.—The spermatozoa are formed in the spermatic tubules which form the main mass of the testis. These tubules are lined by a layer of epithelial cells of cœlomic origin and by the developing sperm-mother cells—the spermatogonia, primary and secondary spermatocytes, and by the spermatids which are transforming into mature spermatozoa. The spermatic tubules are interwoven, but do not appear to communicate with each other except in the region of the hilum of the testis where several may unite together to open into a "straight tubule." These *tubuli recti* open into the vasa efferentia.

The interstices between the spermatic tubules are filled by a variety of elements, chiefly by the blood vessels and lymphatics and by connective tissue. Certain large glandular cells are also present which have been termed "interstitial" and to which many physiological functions have been ascribed.

The testes usually exhibit marked seasonal variation in size. This variation is largely due to the distension and growth of the spermatic tubules at the approach of the breeding season. It occurs also in some mammals with a restricted breeding season, especially Rodents, but is more often absent, as in man.

The testes of Fishes, Amphibians and Sauropsidans are attached by mesorchia to the dorsal body wall in the kidney region. During development they move backwards and downwards in mammals. They remain within the body cavity in

Monotremes and many Eutheria, such as Hyrax, Elephas, and most Edentates. They descend through the inguinal canals into the scrotal sacs in other forms. This descent into the scrotum may be temporary, during the breeding season, as in Rodents and Insectivores, or it may take place early in development and be permanent, as in many forms, including man. The testis is accompanied by the epididymis to which it is closely attached. The descent results in a portion of the mesentery, with the vascular and nervous supply, being drawn out into a long strand or spermatic cord. The inguinal canal becomes constricted, after the testis has passed through, and prevents its return in forms in which the descent is permanent.

The manner in which the descent is effected is far from certain. During descent the gubernaculum shortens and the testis, etc., pass through the inguinal canal into the scrotum, carrying with it an enveloping sac-like extension of the peritoneum, the tunica vaginalis. Whether the descent is effected by an active shortening of the gubernaculum, or whether the decrease in length of this ligament is an effect, not the cause, of the descent, is still in doubt.

The spermatozoa of all vertebrates resemble each other in essential structure. They are produced in enormous numbers and the wastage is prodigious in mammals as well as in the more primitive forms.

The Male Genital Ducts.—The spermatozoa escape from the testis into the cœlom in Amphioxus and the Cyclostomes ; and this is undoubtedly the primitive method. The genital ducts of fishes, other than Elasmobranchs, are of a special type, and their morphology is far from being understood. They arise in both sexes by a backgrowth from each gonad of two peritoneal folds which are converted into a peritoneal tube by the union of their margins. These ducts are continuous with the sac-like gonads in some forms, while in others they are more or less reduced and open into the body-cavity by a funnel. The germ-cells escape into the body-cavity in the latter case and thence through the reduced ducts to the exterior.

The male Protopterus has an arrangement of the ducts which may represent an intermediate condition between those of

Teleosts and those of Elasmobranchs and higher vertebrates. A tubular duct is continuous with the testis posteriorly, but is connected by a network of tubules with the mesonephric or Wolffian duct. It can be seen that, if this testis duct lost its connection with the Wolffian duct and acquired an opening to the exterior, a condition would be arrived at similar to that in Teleosts. On the other hand, if the testis duct was reduced so that the testis was directly connected with the Wolffian duct by the tubular network, the arrangement would be similar to that in other vertebrates, which we will now describe. In them the spermatic tubules of the testis become united with the mesonephric tubules or vasa efferentia by a network or rete of fine canals. The spermatozoa escape through these into the archinephric or Wolffian duct, which constitutes the vas deferens, and thence to the exterior. The mesonephros and Wolffian duct retain their excretory function in the Anamnia, but cease to do so during embryonic life in the Amniota and only function as genital ducts in the adult. The anterior portion of the mesonephros then forms the epididymis, and the posterior portion, which is not connected with the testis, forms the rudimentary paradidymis. These structures remain rudimentary or degenerate more or less completely during development in the females of Amniota. Vestiges of the Wolffian duct and rete, as well as of the anterior and posterior portions of the mesonephros, paroophoron, and epoophoron respectively, can generally be detected even in the adult female.

Internal Fertilisation.—The males of many vertebrates possess intromittent or copulatory organs to facilitate, during mating, the introduction of the spermatozoa into the female genital tract. These organs have been evolved separately more than once in vertebrates to meet the need for internal fertilisation and the consequent reduction in the wastage of germ-cells entailed by external fertilisation. Among fishes the Elasmobranchs have developed copulatory organs in the form of paired claspers which are modified portions of the pelvic fins and which flank the cloaca of the male. A few viviparous Teleosts also have the anal fin modified for copulation. These organs are inserted into the oviducts of the female during mating

so that their grooved inner surfaces form a channel for the transference of the seminal fluid. The Amphibia have no true copulatory organs, but in the Urodela and Gymnophiona fertilisation is internal and the spermatozoa are transferred directly from the cloaca of the male to that of the female. Fertilisation is external in the Anura, but the male clasps the female by special pads developed on the thumbs while spawning is taking place. Internal fertilisation is universal among the Sauropsida, in which two different types of copulatory organs are found. Lizards and snakes have paired copulatory sacs opening into the cloaca which can be everted and protruded. The seminal fluid passes down a spiral furrow in the surface of each when everted. The females possess similar organs in a rudimentary state. Chelonians, crocodiles, and many birds have a single penis developed on the ventral wall of the cloaca. It contains erectile tissue and is protrusible and grooved for the conveyance of the seminal fluid. The penis in the female is represented by a small clitoris. The penis of mammals appears to be derived from a structure resembling that of crocodiles. The Monotremes have a well-developed protrusible penis attached to the ventral wall of the cloaca. This penis contains erectile tissue and is pierced by a canal through which the semen is emitted. The urogenital canal opens into the cloaca at the base of this organ. The penis is separated from the cloaca in the Marsupials and, when retracted, is enclosed in a prepucial sheath which opens on the surface of the body between the anus and the scrotum. The urogenital canal does not communicate with the cloaca, but opens at the apex of the penis, which contains erectile tissue. The penis of the Eutheria is essentially similar to that of the Marsupials, but is situated in front of the scrotum.

Accessory Glands.—Certain glandular organs are developed in connection with the male genital ducts. The posterior portion of the Wolffian duct in Elasmobranchs is dilated into wide, thin-walled vesiculæ seminales, and a pair of long, narrow sperm-sacs open into the urogenital sinus. Among the Urodele Amphibia numerous glands open into the cloaca and secrete a gelatinous substance which unites the spermatozoa

PLATE I

Mammalian Ovaries

1. Left ovary of Platypus (Ornithorhynchus) showing two corpora lutea and many follicles projecting from the surface. × 7 approx. (From Hill and Gatenby [111].)

2. Human ovary from a young adult, showing the smooth contour. A large follicle is present beneath the furrow at the left-hand lower corner of the figure. The other scars were formed by the rupture of follicles at previous ovulations. Part of the broad ligament is cut away at the top of the figure to show the Fallopian tube and the fimbria to the left of the ovary. × 1·3.

PLATE I

1

2

into packets or spermatophores. Many Sauropsida also have accessory genital glands developed in the wall of the cloaca. Among the mammals the accessory genital glands reach their highest development. A pair of glandular sacculated vesiculæ seminales open into the urethra together with the vasa deferentia at the base of the bladder. Ampullary, prostate, urethral, and Cowper's glands are also present, together with various superficial glands associated with the external genital organs. The secretion of these glands appears to furnish an important ingredient of the seminal fluid and is believed to play a part in the activation of the spermatozoa. The secretion of these glands in Rodents and Insectivores coagulates after emission and forms a hard plug in the vagina which prevents the premature escape of the seminal fluid.

The Ovary.—It has been stated that the peritoneal investment of the vertebrate ovary persists throughout life as the germinal epithelium. Beneath this covering is a thin fibrous connective tissue layer, the tunica albuginea, which never assumes the proportions of this layer in the testis. The ovary may be divided into a medulla and a cortex. The medulla contains blood-vessels, lymph channels, etc., separated by trabeculæ of fibrous connective tissue. The cortex contains the oocytes in various stages of development, each invested by a follicle. Between the follicles is the stroma, composed of connective tissue, containing epithelial elements and often various kinds of glandular elements which have been termed indiscriminately "interstitial cells." The ovary exhibits marked hypertrophy at the approach of the breeding season in most forms. It assumes then the appearance of a bunch of grapes in forms which produce large eggs, such as the Elasmobranchs, Sauropsida, and Monotremes (Pl. I, Fig. 1). In forms with small eggs, such as the higher mammals, its contour is more smooth and even (Pl. I, Fig. 2).

The ovaries in many Teleosts have become completely enclosed in cœlomic pouches. They are consequently in the form of hollow sacs and are said to be cystovarian. They are exposed in the body cavity, and the mature oocytes burst through the germinal epithelium into it in all other forms. The ovaries

of some forms, such as the Amphibia and Monotremata, appear sac-like owing to the large development of the lymph spaces in the medulla. This appearance is obviously deceptive and is not to be confused with the true cystovarian condition in which the ovarian cavity is of cœlomic origin.

The ovaries are attached to the dorsal body wall by a mesentery or mesovarium, inserted along the hilum. Rudimentary structures homologous to the epididymis and paradidymis of the male are usually present in the region of the ovarian hilum, and are known as the epoophoron and paroophoron respectively.

The ovaries of mammals, although conforming to this general plan, are more complicated than those of other forms. In the Monotremes the oocytes of all sizes are confined to a narrow, folded, cortical region which constitutes the main bulk of the ovary. The medulla is full of large lymph sinuses, separated by connective tissue trabeculæ, which impart to it a characteristic spongy texture. The mature oocytes are liberated from their follicles by the rupture of the wall. After their liberation, a glandular corpus luteum, rather larger than the mature oocytes, is formed in each empty follicle by the ingrowth of the cells composing its walls. The corpus luteum of the Monotreme is essentially similar in structure to that of the higher mammals, and presumably is an endocrin organ concerned with the control of gestation and lactation. Many of the oocytes at all stages of development undergo degeneration (atresia), as is also the case with Sauropsida and the higher mammals. In the latter these atretic oocytes are absorbed *in situ*, but in the Monotremes the contents of the atretic follicles are discharged directly into the medullary sinuses of the lymphatic system.

The ovaries of the higher mammals are much smaller in proportion than those of the Monotremes and are smooth or slightly lobed. Both ovaries of the pair are functional and are equal or sub-equal in size. Only mature follicles and large corpora lutea project from their surfaces, and then only very slightly. The ovaries themselves are generally oval in outline and partially flattened. They are firm in texture

on account of the development in them of a considerable amount of fibrous connective tissue. The central medullary region is small and contains the vessels and small lymph channels. The cortex is thick and dense, and composes the major portion of the ovary. It is surrounded by a thin fibrous coat, the tunica albuginea, which is covered on the outside by the germinal epithelium. The oocytes are always very small, but the ripening follicle becomes distended by a fluid-filled cavity, the antrum, and attains a considerable size before rupturing. The characteristic mammalian follicle is known as the Graafian follicle, a term which should be strictly confined to follicles with an antrum, and consequently should not be used for those of Sauropsida or Monotremata.

A corpus luteum is formed in the ruptured follicle and grows larger than a mature follicle. The ova of all higher mammals are of approximately the same size and bear little or no relation to the size of the whole body. The mature follicles and corpora lutea, on the other hand, vary very considerably in relation to the bulk of the animal. The mature follicle of the mouse, for instance, measures about 0·5 mm. in diameter and the corpus luteum about 0·7 to 0·9 mm. in diameter, while the whole ovary weighs about 5 mgrms. In the cow the whole ovary weighs 10 to 12 grms. approximately, while a mature follicle may measure as much as 1·5 cms. in diameter and the corpus luteum attains a size of about 2 cms. in diameter. This phenomenon is of considerable theoretical interest, as will be seen later in considering the function of the mammalian Graafian follicle. The tissue between the follicles is composed of connective tissue stroma in which are embedded glandular-looking cells. These cells have been termed " interstitial " by many workers. This term is well avoided, however, since it has been applied to any inter-follicular cell which is not obviously a connective tissue element and which has a glandular or adipose appearance, and consequently includes many different categories of cells, which are not homologous. The ovaries are attached to the dorsal or dorso-lateral body wall by the broad ligament. The insertion of the broad ligament on the ovary has become much constricted so as to form a narrow

hilum, where the ovarian vessels and nerves enter. In some mammals the ovaries are freely exposed in the body cavity, in others they have been surrounded by a more or less complete ovarian capsule, including a periovarian cœlomic space, by the growth of folds of the peritoneum around them. During development the ovaries, at first ventral to the anterior part of each kidney, move backward and sometimes ventrally as well. In the Rodents the ovaries in the adult are situated immediately posterior to the kidneys, but in other forms, as, for instance, in man, they have descended still further and are situated in the pelvis, some distance posterior to them.

Unilateral Development of Gonads.—Although the gonads are morphologically paired organs in all vertebrates a number of forms exhibit unequal development on the two sides of the body. It has been seen that the gonads form a row on each side of the body in Amphioxus. This is not so, however, in the allied genera Heteropleuron and Asymmetron, in which there is only a single row, situated on the right side. The single gonad of the Cyclostomes is median in Petromyzon and probably represents those of both sides fused ; in Myxine it is on the right side, that on the left probably being undeveloped. The testes of all higher forms are functional on both sides, but the ovaries in many Elasmobranchs and in Birds and Monotremes are only fully developed on one side. The right ovary is functional and the left rudimentary in Scyllium, Galeus, Mustelus, Zygæna, Carcharias, Pristiophorus, and several other Elasmobranchs. The left is the chief in Birds, but the right is rudimentary, rarely functional. The left ovary is the largest in Ornithorhynchus, while it is sometimes the left and sometimes the right which is functional in Echidna (Hill and Gatenby [111]). The unilateral arrangement of the ovaries in these forms, all of which produce very large ova, may be an adaptation to avoid the overcrowding which would occur if both ovaries were fully developed and contained large oocytes. The value of this adaptation is obvious in birds, where the simultaneous formation of two hard-shelled eggs would be fatal.

The Oviducts.—The oviducts of all vertebrates above the

Cyclostomes, with, as has been mentioned, the exception of the Teleosts, are developed from the Müllerian duct. This duct is developed in the Elasmobranchs by a process of longitudinal splitting from the Wolffian duct. It develops in other forms close to, but separate from, the Wolffian duct by the back-growth of the apex of a funnel-shaped depression of the peritoneum in front of the gonad. The Müllerian duct remains vestigial in the male.

The oviduct consists essentially of an outer peritoneal investment, a muscle layer, and an inner mucous layer rich in glands. The glands of the mucosa secrete the albumen and shell.

The oviducts of the Elasmobranchs, Amphibians, Sauropsida, and Monotremata resemble each other closely. They consist each of (1) a peritoneal funnel, which is ciliated and often fimbriated to assist in engulfing the ova, (2) a glandular portion which secretes the albumen and is often much convoluted, (3) an oviducal sac in which the egg is retained until laying.

The oviducts of Marsupials and Eutherians have become specially adapted for the maintenance of the young. Four distinct regions can be distinguished in them: (1) The ostium or funnel, (2) a narrow tubular portion—the Fallopian tube, (3) a large uterus, with well-developed muscular walls and a thick glandular mucosa, (4) a wide, thin-walled vagina opening into the vulva or urogenital orifice. Fertilisation is effected in the Fallopian tubes. The uteri of the two sides may be quite separate (mouse), partly united (marsupials), or entirely fused, with a single cavity (man). The development of the young takes place in the uterus, the mucosa of which contributes to the formation of the placenta. The vagina is usually a single median organ, but is paired in a few marsupials.

Albumen and Shell Formation.—The eggs of Amphioxus, the Cyclostomes and Teleosts have no other covering than the vitelline membranes formed around them in the ovary. The eggs of Elasmobranchs and all higher vertebrates are surrounded by a layer of albumen, and often by a shell, formed by the oviduct. The large eggs of the Elasmobranchs are surrounded by a considerable quantity of albuminous mucilage and by a horny shell. Amphibians' eggs have no shell, but

the albumen swells tremendously after laying owing to the absorption of water. The eggs of all Sauropsida are richly supplied with albumen. The shell is leathery in some reptiles but is calcified in others and in birds.

The mature egg of Ornithorhynchus measures about 3 mm. in diameter. A thin layer of albumen and a leathery shell are formed around it by the oviduct after fertilisation (Fig. 2).

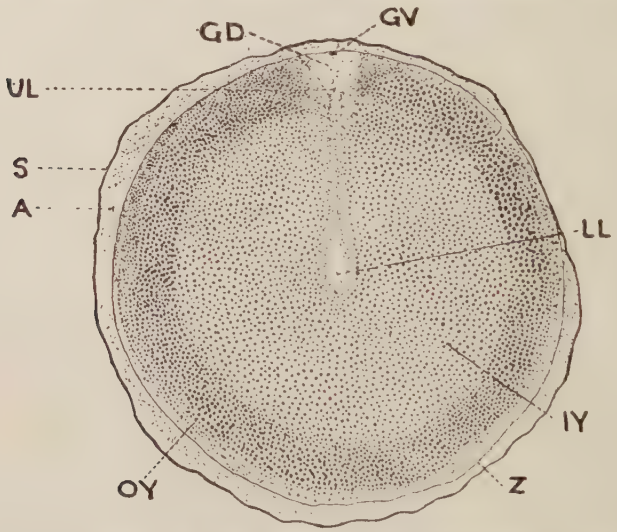


FIG. 2.—A mature egg of the Platypus (Ornithorhynchus). A, albumen; GD, germinal disc; GV, germinal vesicle; IY, inner zone of yolk; OY, outer zone of yolk; LL, lower end of latebra; S, leathery shell membrane; UL, upper end of latebra; Z, zona pellucida. ×16. (From Gatenby [84].)

It then measures about 4 mm. in diameter. A large part of the development takes place in the oviduct, the secretion of which is absorbed by the egg. Owing to this absorption it attains a size of about 16 to 18 mm. long by 14 to 15 mm. in diameter. Two eggs are laid at the same time and become cemented together after laying by a sticky deposit formed on the shell by the oviduct. Hartman [106] has described the occurrence of a thin layer of albumen and a shell membrane, formed by the Fallopian tube, around the tubal egg of the Opossum. The eggs of higher mammals have no shell, but a slight albuminous envelope is secreted around them by the Fallopian tube.

The Œstrous Cycle.—Adult female mammals during the breeding season exhibit a co-ordinated series of changes in the ovary, uterus, vagina, and mammary glands which is known as the œstrous cycle (see Marshall [152]). This cycle culminates in the period of heat or œstrus during which alone most mammals will copulate. Œstrus is followed by metœstrus, then by diœstrus, pro-œstrus, and finally œstrus again. Some mammals continue repeating this cycle throughout the year (man), while others, with a limited breeding season, remain inactive in this respect (anœstrus) for the greater part of the year. When pregnancy follows copulation the characteristic changes in the uterus, preparatory to the growth of the embryo and the formation of the placenta, supervene and the non-pregnant cycle is upset. In some mammals œstrus is always followed by the initial stages of such a process. These soon cease and the return to the normal is often accompanied by considerable disruption of the uterine mucosa and extravasation of blood. This pseudo-pregnant phase is a regular feature of the cycle in the human, but only occurs after copulation in many animals (rabbit, mouse, etc.). Menstruation in the human is almost certainly the disruptive stage which terminates this pseudo-pregnancy. These uterine and vaginal changes are accompanied by changes in the ovaries. During pro-œstrus or œstrus ovulation occurs and is followed by the formation of the corpus luteum. The corpus luteum degenerates before the next œstrus is due unless pregnancy supervenes, when it grows slightly and persists until about the time when the young are born. The mammary glands undergo a slight cyclic enlargement corresponding with the uterine changes, and they undergo marked hypertrophy during pregnancy, in preparation for the ensuing lactation period. These elaborate changes appear to be controlled by the ovary, as will be seen later.

Placentation.—The young in viviparous Teleosts develop either in the follicles or in the cavity of the ovary. They appear to absorb a certain amount of nourishment from the ovary, but no secondary connection with it is formed. The viviparous habit appears to have arisen separately in several families, which

are not closely related. It is of an entirely different type from the development in the oviduct in Elasmobranchs and mammals.

The embryo in viviparous Elasmobranchs completes part of its development in the dilated uterine portion of the oviduct. The intra-uterine embryo escapes after a time from the egg-shell (Daniel [64]). The mucosa of the uterus is thrown up into numerous long villi with a rich vascular supply. The developing embryo derives nutriment from the maternal blood circulating in these. This is effected in some forms by absorption through the thin shell, in others by the formation of a yolk-sac placenta through the direct attachment of yolk-sac villi with the maternal villi. The details of intra-uterine development vary considerably in different Elasmobranchs, but it is evident that a high degree of adaptation has been attained.

All mammals except Monotremes have highly specialised placentæ. The placenta in some Marsupials is formed first by the yolk-sac. Traces of a similar yolk-sac placenta can be found in early stages of some Eutheria. The yolk-sac placenta is rapidly replaced by the definitive placenta formed by the chorion with or without the help of the allantois. The placenta attains considerable dimensions during gestation and is more or less completely shed at parturition. The remnants which are not shed are speedily reabsorbed. The structure of this organ is highly specialised and characteristic of each order.

Lactation.—The young in Marsupials are transferred by the mother or else crawl after birth to a special pouch or marsupium in the skin of the abdomen. The young in Eutheria are much more developed when born. All mammals nourish the young for some time after birth with milk secreted by mammary glands situated on the ventral surface of the body. These open by numerous small pores on the mammary area in Monotremes and the young suck the milk from the long hair growing on this area. The openings of the mammary glands are situated on paired nipples in all other mammals. The nipples are restricted to the marsupium in Marsupials, but are arranged primitively in two rows extending from the armpit to the groin in higher mammals, although in most forms only some of the glands are persistent.

CHAPTER II

SPERMATOGENESIS

The essential fact in the production of the gametes is the reduction of the somatic or diploid number of chromosomes to one-half, so that the diploid number will result again from the fusion of the spermatozoon and ovum at fertilisation. This is effected at the meiotic or reduction division which follows on the elaborate phenomena of the meiotic prophase. The prophase changes are essentially similar in the two sexes, but exhibit minor differences connected with the minute and motile character of the spermatozoon as contrasted with the large and immotile nature of the ovum.

The differences in the cytoplasmic processes leading up to the production of the spermatozoon and ovum are very great, the one resulting in the formation of highly specialised structures of the flagellate sperm and the other in the intense accumulation of yolk characteristic of the egg. So fundamental are these differences that the cytoplasmic phenomena of spermatogenesis and oogenesis appear to have little in common.

The spermatozoa of all vertebrates are uni-flagellate, like those of most invertebrates. They exhibit various modifications in the different vertebrate groups, but all conform to a common plan. The modifications appear to have had little effect on the evolution of the vertebrates.

The ova of vertebrates, other than mammals, exhibit a progressive modification in the direction of increase in size. This has had a profound effect on the evolution of the vertebrates and was correlated with the chief adaptations which have led up to the elaborate reproductive system of the Mammalia. We will proceed, with this in mind, to a more

detailed examination of the formation and structure of the spermatozoa and, in the next chapter, of the ova.

Spermatogenesis.—The germinal epithelium lining the spermatic tubules in vertebrate testes consists of germ-cells and Sertoli cells. The latter are epithelial cells which have assumed the special function of supplying nutriment to the sperms, which become attached to them during the later stages of their formation.

Spermatogenesis is a process which goes on continuously in the testes throughout the breeding season. It proceeds from puberty until old age in man and those animals which breed all the year round. The germ-cells are, therefore, in all stages of transformation into mature spermatozoa in every tubule. The Sertoli cells are attached to the walls of the tubules and the germ-cells are arranged with the earlier stages attached to, or near, the wall, and the later stages adjoining the lumen. The mature spermatozoa are free in the lumen itself.

The spermatogonia represent the first stage in the production of the spermatozoa. These cells undergo an indefinite number of divisions and are to be found in the testis at all seasons. Ultimately each spermatogonium divides into two primary spermatocytes which grow and then undergo the first maturation division, each producing two secondary spermatocytes. Each secondary spermatocyte undergoes the second maturation division and produces two spermatids. The spermatids transform into spermatozoa directly, without further division, the term " spermateleosis " being used for this process. Each primary spermatocyte, consequently, gives rise to four spermatozoa. During the maturation divisions the diploid number of chromosomes is reduced to the haploid, to provide for the subsequent doubling which results from the fusion of the ovum and spermatozoon at fertilisation. This reduction is effected by the separation of the two members of each pair of similar chromosomes at one, usually the first, of the maturation divisions. This division is the reduction, meiotic or heterotypic division. At the second maturation division the chromosomes split into two, one-half going to each daughter

cell. This division is similar, therefore, to a normal mitosis except that there are only the haploid, instead of the diploid, number of chromosomes. The second maturation division is known, in consequence, as the homotypic division. Both the heterotypic and the homotypic divisions of the germ-cells are characteristic and require more detailed description. The account, now classical, of spermatogenesis in Lepidosiren given by Agar [1] furnishes a good example.

The Nucleus in Spermatogenesis.—Lepidosiren is specially suited to cytological study on account of the large size of the nuclei and the sharp outlines of the chromosomes in fixed material. The diploid number of chromosomes is thirty-eight. The nineteen chromosomes in the gametes differ in size and can consequently be distinguished from each other at all stages in which the individual chromosomes are visible.

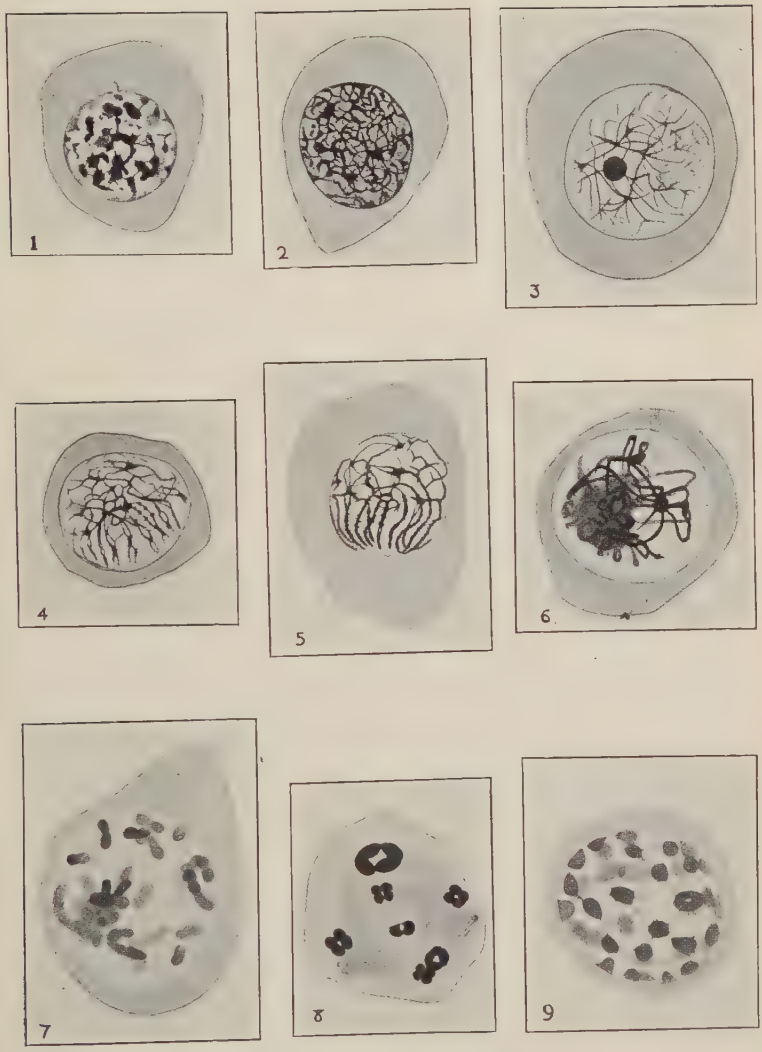
The spermatogonial nucleus is characterised by the large irregular blocks of chromatin connected by finer strands, which give it a coarser appearance than the resting spermatocyte nucleus. The earlier generations of spermatogonia are larger and the nuclei are often filled by a densely staining, diffuse substance which obscures the structure. These enter on a period of more rapid multiplication, after which the spermatogonia are small and the nuclei contain no diffuse substance. The last generation of these later spermatogonia gives rise to the primary spermatocytes. These are distinguished by entering on a growth period during which the nuclei are in a resting condition. At the end of this period the spermatocytes are larger than any of the spermatogonia and the nuclei have a characteristic structure. The chromatin is arranged on a very fine network, never aggregated into large blocks, as in the spermatogonia, and there is a single large nucleolus. The primary spermatocyte in this condition is ready to enter on the prophase changes leading up to the first maturation or heterotypic division. These prophase changes are divided conveniently into five stages, for which the following names, based on those used by de Winiwarter [232], are now in general use. They are: leptonema, zygonema, pachynema, strepsi-

PLATE II

The Nucleus during Spermatogenesis in Lepidosiren paradoxa

1. Spermatogonium. 2. Primary spermatocyte at the end of the growth period. 3. Leptotene stage of the prophase of the heterotypic division of the spermatocyte. 4. Transition of the leptotene into the zygotene stage. 5. Transition stage between zygotene and pachytene. The chromosomes are arranged in the bouquet form. 6. Synizesis. 7. The cell at the close of synizesis showing shortening and thickening, and complete separation of the univalent chromosomes, which are becoming transversely constricted. 8. Some of the bivalents, after the pairing of the chromosomes is complete, arranged on the spindle of the heterotypic division. 9. The resting stage showing the thirty-eight chromosomes arranged immediately beneath the nuclear membrane. All figures ×950. (From Agar[1]).

PLATE II



nema or diplonema, and diakinesis (Pl. II). The appropriate adjectives are formed by substituting " tene " for the suffix " nema " with the exception of the last, which becomes " diakinetic."

The resting nucleus of the primary spermatocyte gradually transforms into the leptotene stage in which the chromatin is arranged in very long, fine threads, the complicated coils of which fill the nucleus. The disordered arrangement of the chromatin threads in the leptotene nucleus soon gives way to the more definite arrangement observed in the zygotene stage. The threads become arranged in a parallel manner for a part of their length at one pole of the nucleus, probably that next the centrosome. They then become twisted together, according to Agar, and fuse in pairs. This process begins at the pole and extends away from it. This stage has excited much controversy, since two incompatible views are held concerning the members of each pair of fusing threads. Agar and an ever-increasing number of cytologists consider that each thread is a single chromosome and that the pairs consist of two homologous chromosomes side by side. The other view is that the pairs are single chromosomes split longitudinally. It is impossible in this book to consider the pros and cons of the two theories. The former will be adopted in consequence, since it is the more generally accepted and has proved the more useful working hypothesis.

The fusion of the homologous chromosomes in pairs is known as syndesis. It proceeds until all the threads are fused in pairs throughout their length. The chromosome threads have, at the same time, shortened continuously and are now in the form of horseshoe-shaped loops with the free ends at the pole of the nucleus next the centrosome. This is the pachytene nucleus, often spoken of from its characteristic appearance as the *bouquet stage*. It is clear that since the homologous pairs of chromosomes have fused and are each represented by a single loop there should be the haploid number or nineteen of such loops. Agar found that this actually was the case.

The pachytene stage is immediately followed by the strepsitene or diplotene in which the conjugating chromosomes

split apart, only remaining attached at their ends. Each pair of chromosomes thus forms a long loop. This stage is complicated in Lepidosiren by a marked contraction of the chromatin towards one side of the nucleus, which obscures the individual loops. This contraction, known as synizesis, is a common, although not universal, phenomenon of the meiotic prophase of many animals and plants, although the degree and duration vary considerably. It is of comparatively short duration in Lepidosiren. The chromosomes forming the rings in the strepsitene stage gradually break apart, thus restoring the diploid number of chromosomes in diakinesis.

This separation of the members of the bivalents, or fused pairs of chromosomes, during diakinesis in Lepidosiren is by no means common in other animals. A resting stage, less marked, but apparently comparable to that in oocytes, may be intercalated in diakinesis.

The chromosomes, as a result of the continuous shortening which has been proceeding since the onset of the leptotene stage, are seen to be in the form of short, thick rods arranged close under the nuclear membrane in diakinesis. The nuclear membrane then undergoes dissolution and the achromatic figure appears. The thirty-eight single chromosomes of the diakinetic nucleus then again come together in homologous pairs. These bivalents become arranged on the spindle and at the metaphase one member of each goes to one daughter-nucleus, and the other member to the other daughter-nucleus. Each secondary spermatocyte thus gets the reduced number of nineteen chromosomes.

The secondary spermatocytes enter on the homotypic division as soon as the heterotypic division is complete. There is no resting stage between the two divisions. The homotypic division resembles an ordinary somatic mitosis except that only the reduced or haploid number of chromosomes are present. The chromosomes split longitudinally and divide equationally in a normal manner, needing no further description.

The reduction divisions in the spermatogenesis of other vertebrates exhibit minor variations from those described, but conform to the same general scheme.

The nucleus of the spermatid during spermateleosis becomes compressed and compacted to such an extent that no structure can be distinguished in the dense mass of chromatin which forms the nucleus of the mature spermatozoon.

The Cytoplasm in Spermatogenesis.—The cytoplasmic processes which contribute to the formation of a mature spermatozoon are almost as complicated as those in the nucleus. Moreover, this branch of work has not received the same amount of attention, since the more important cytoplasmic organs concerned can only be demonstrated by special modern methods. The centrosome, Golgi bodies, and mitochondria in particular play an important part, as will be seen in the following account of the spermatogenesis of Cavia (see Pl. III) based on the work of Gatenby and Woodger [90] and other workers [86, 88, 89, 197].

The centrosome in the resting primary spermatocyte is situated at one side of and close to the nucleus. It is surrounded by the archoplasm in the form of a sphere of dense protoplasm. The zone of the archoplasm contains a large number of small discrete granules, the proacrosomic material, which will ultimately form the acrosome or head-cap of the spermatozoon. The outer zone of the archoplasm does not contain these granules. The numerous Golgi bodies, in the form of small semi-lunar rods, lie upon the outer surface of the archoplasm. The mitochondria are in the form of little spheres, all about the same size, scattered throughout the cytoplasm. A chromatoid body also is frequently found in the cytoplasm, but its function is unknown.

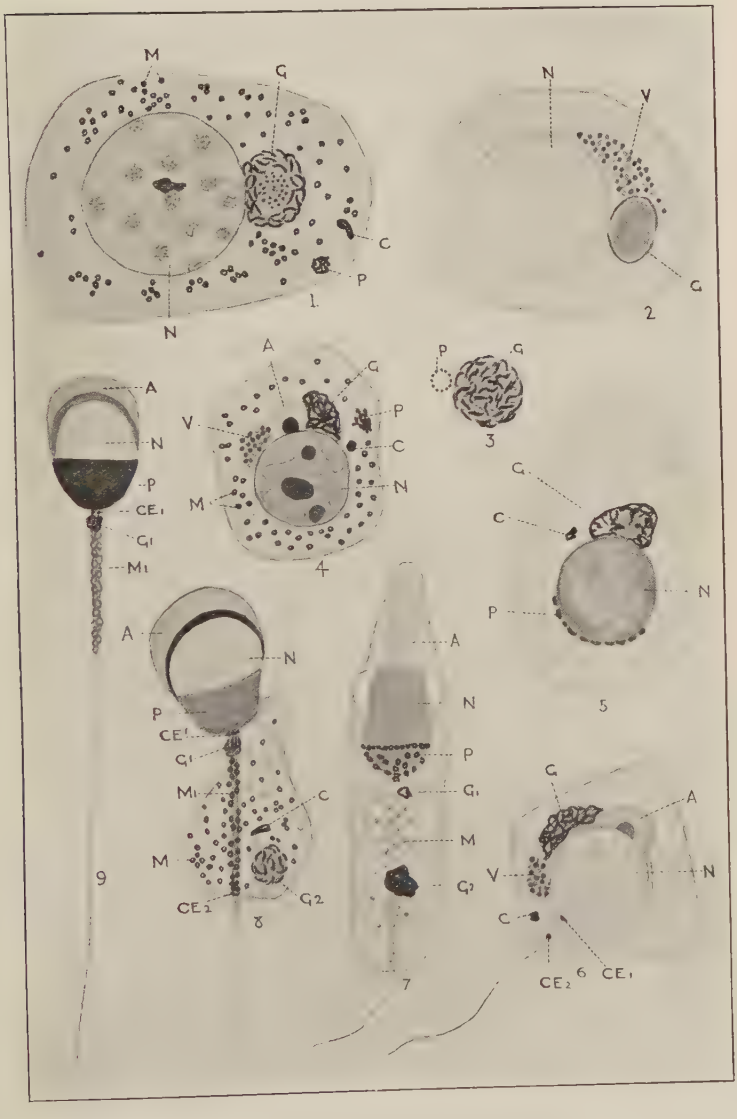
During the maturation divisions the Golgi bodies, which have become detached from the centrosphere and scattered in the cytoplasm, group themselves around the aster and are consequently sorted out in approximately equal numbers to the two daughter-cells. The proacrosomic granules also become scattered and, with the mitochondria, appear to be approximately divided between the two daughter-cells. The behaviour of the chromatoid body is uncertain, but since it occurs in the majority of spermatids it is probable that it divides also.

PLATE III

Cytoplasmic Structures during Spermatogenesis in the Guinea-pig (Cavia)

1. Spermatocyte showing the Golgi bodies (G) surrounding the archoplasmic sphere in the centre of which is the centrosome containing two centrioles. The pro-acrosome granules are enclosed in the archoplasm and lie around the centrosome. The cluster of post-nuclear granules (P), the chromatoid body (C), the scattered mitochondria (M), and the nucleus (N) are also shown. 2. Living spermatocyte stained with neutral red showing the neutral red vacuoles (V), the archoplasmic sphere surrounded by the Golgi apparatus being close to the nucleus. 3. The Golgi apparatus of a spermatocyte with the post-nuclear granules attached. 4. Spermatid showing the formation of the acrosome (A) by the Golgi apparatus. 5. Spermatid showing the post-nuclear granules forming a cup around the nucleus at the opposite end to the acrosome. 6. Spermatid showing the tail filament and the associated proximal (CE1) and distal (CE2) centrioles. The Golgi apparatus is beginning to move away from the acrosome. 7. Later spermatid beginning to lengthen, showing the middle-piece Golgi bead (G1) and the residual Golgi apparatus (G2). 8. Nearly ripe spermatozoon with the middle-piece bead attached. Some of the mitochondria (M1) are becoming arranged around the tail filament between the proximal and distal centrioles to form the middle-piece. 9. Mature spermatozoon after the middle-piece bead has been sloughed off. About 1,300 diameters magnification. Somewhat diagrammatic. (After Gatenby, etc. [86, 88, 89, 90].)

PLATE III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The arrangement of the cytoplasmic structures in the newly formed spermatid is similar to that in the spermatocyte, except that the centrosome is in the cytoplasm, but outside the archoplasmic sphere. Soon a small vacuole forms around each proacrosomic granule in the archoplasm. These vacuoles gradually run together and their contained granules fuse to form two or three large granules each surrounded by an archoplasmic vacuole. The centrosome has divided into two, and the axial filament begins to grow out from them at this stage. The Golgi bodies and archoplasm move away from the centrosome to the opposite or anterior pole of the cell, where they become closely applied to the nuclear membrane. The proacrosomic granules have all fused by this time into a single large bead, the proacrosome, surrounded by a vacuole. An inner and an outer zone can now be distinguished in the proacrosome, which moves through the cytoplasm and becomes directly applied to the nuclear membrane to form the acrosome. The Golgi bodies and archoplasm remain in contact with the acrosome for a time, during which it grows rapidly. Then they break away and drift towards the tail of the spermatid, while the acrosome completes its development alone.

Elongation of the spermatid now begins. During this process the head centrosome becomes attached to the posterior end of the nucleus by the post-nuclear granules (Gatenby and Wigoder [88]). These granules become fused to form a cup closely investing the nuclear membrane at the posterior end, thus forming a fulcrum for the attachment of the tail. The middle-piece is formed by the arrangement of some of the mitochondria and of a small piece of the archoplasm, with some Golgi bodies attached to it, around the axial filament. The remainder of the mitochondria, the Golgi bodies, and the archoplasm, together with the chromatoid body and almost all the cytoplasm, pass back into a bead attached to the middle-piece. Only a thin sheath of cytoplasm remains around the middle-piece and the greater part of the length of the axial filament. This middle-piece bead remains attached to the mature spermatozoon of Cavia and many other animals for a considerable time, but is almost certainly lost before fertilisation.

The middle-piece bead in other mammals, such as the mouse, is sloughed off the maturing spermatozoon.

Structure of Mature Spermatozoa.—The mature spermatozoon of any vertebrate thus exhibits three main regions, a head, middle-piece, and tail, each of which may be divided conveniently into two. Thus the head consists of the acrosome and nucleus, the middle-piece of the neck and middle-piece proper, and the tail of a main-piece and an end-piece. The shape, size, and relative proportions of these parts exhibit great variation in different species, although the functional significance of the various forms is practically unknown.

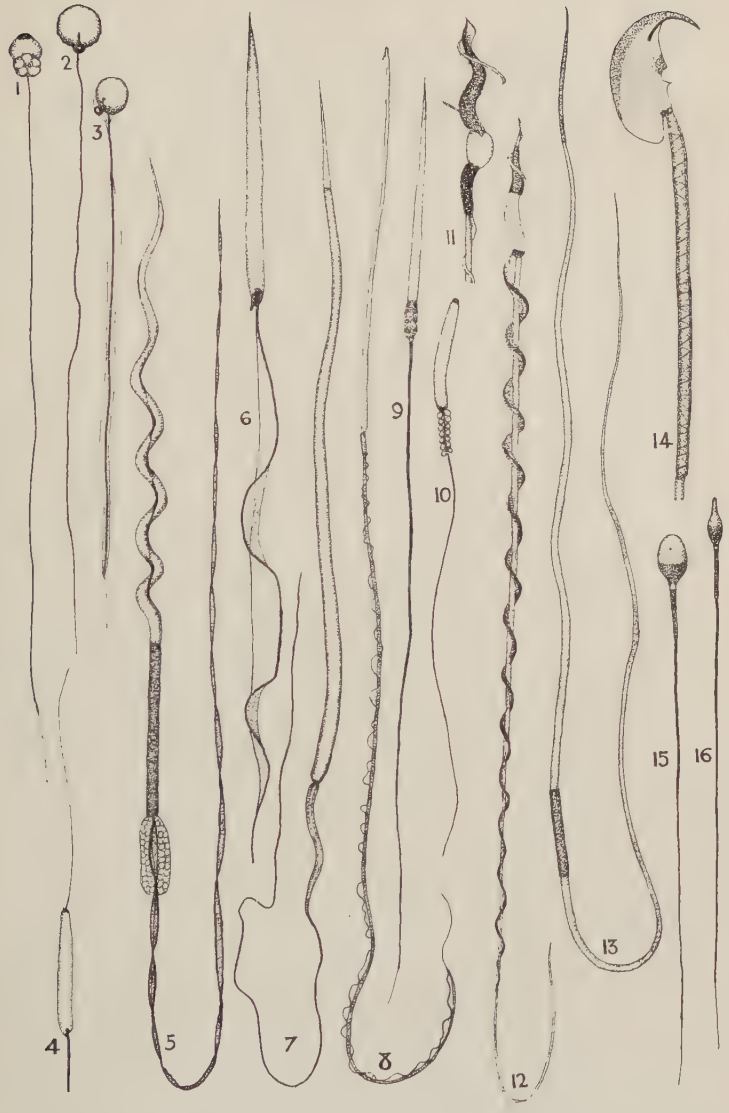
The acrosome is attached to the anterior end of the nucleus and both are invested by an extremely delicate film of cytoplasm. Special techniques have revealed that this film is often in the form of fibrillæ wound spirally around the nucleus and possibly acting as supporting structures (Koltzoff [134]).

The neck is a short region, frequently ill-defined, which is occupied by the basal or proximal centriole or centrioles from which the axial filament arises. It is attached to the head by the post-nuclear apparatus and may be defined as the region which unites the head and tail. It is frequently devoid of a mitochondrial sheath. The middle-piece proper is the region immediately behind the neck between the proximal and distal centrioles, which are generally some distance apart. It consists of the axial filament surrounded by a mitochondrial sheath, often arranged in a spiral. It is known, in some mammals at least, to contain some Golgi bodies.

The axial filament is continued to the tip of the tail. This filament can be shown by maceration to consist of fine fibrillæ. The cytoplasm forms a thin investing membrane around the middle-piece and the main-piece of the tail. It does not extend over the end-piece of the tail, however, where the axial filament appears to be naked. An undulating membrane is often attached to the axial filament along the entire length of the main-piece of the tail.

Types of Vertebrate Spermatozoa.—Our knowledge of the structure of the sperms of various forms of animals is

PLATE IV



TYPES OF VERTEBRATE SPERMATOZOA

1. Amphioxus. 2. Salmo. 3. Perca. 4. Petromyzon. 5. Raja. 6. Bufo. 7. Rana. 8. Salamandra. 9. Anguis. 10. Crex. 11. Fringilla. 12. Turdus. 13. Echidna. 14. Mus. 15 and 16. Man (in plan and profile respectively). (Modified from Retzius[200].)

largely due to the work of Retzius [200] and Ballowitz, [10] which has been summarised by Wilson [230].

The spermatozoa (Pl. IV) of Amphioxus are very primitive and resemble those of many invertebrates. The head is almost spherical and the middle-piece is short and rounded and appears to consist of a ring of about five mitochondrial spheres surrounding the base of the axial filament. The sperms of the Teleosts resemble this type also; but in some cases the flagellum bears a simple undulating membrane.

The sperms of Elasmobranchs resemble the amphibian more closely than the teleost type. The head is elongated and often spirally twisted. The middle-piece is short with a spiral arrangement of the sheath. The tail consists of two or three filaments in a spirally twisted membrane.

Among the Amphibia the simplest type of sperm is found in the Anura. The head in most of these is very elongated, and while some appear to have no true middle-piece, proximal and distal centrioles being together, others have one, short but distinct. The tail of the sperm in the toads consists of two filaments united by an undulatory membrane, giving the sperm in some cases a superficial resemblance to a trypanosome. The head of the sperm in Urodeles is also elongated, and in many the acrosome is very long, fine, and barbed. The neck is well marked and the true middle-piece is very long and constitutes the entire tail except for the short end-piece. An undulating membrane is attached along the length of the middle-piece.

The sperms of reptiles and the majority of birds are of a simple type with the three chief regions clearly defined. The head is long and straight and the middle-piece exhibits a spiral formation of its mitochondrial sheath. The middle-piece in some forms is elongated to form the major part of the flagellum, as in Urodeles. The sperms of the passerine birds are more complex. The nucleus is oval in shape and about one-third or one-quarter of the length of the acrosome in many cases. A conspicuous spiral membrane on the acrosome gives the head the appearance of a corkscrew. This membrane is bounded by a filament which is probably continuous with one surrounding the nucleus. The middle-piece is short and distinct.

The main-piece of the tail has one or two filaments wound in a spiral around the axial filament. The end-piece is long.

The sperms of Echidna, unlike those of higher mammals, are sauropsidan in character ; an interesting fact which might have been expected. The head is very elongated, being almost as long as the tail, and the middle-piece is distinct. The sperms of other mammals, though very varied, exhibit a general similarity to each other. The head is generally oval and flattened, sometimes spoon-shaped when viewed from the side, as in rodents. The middle-piece is well developed and frequently exhibits a spiral arrangement of the mitochondrial sheath, while the tail is simple and always devoid of a fin membrane.

This brief outline serves to show that the general plan of structure of the spermatozoa is common to all vertebrates, although each group has peculiarities of its own. It is interesting that the spermatozoa of Elasmobranchs resemble those of the Amphibia comparatively closely, while those of the Teleosts are widely removed in structure, and that those of all mammals, excepting Monotremes, are very characteristic and easily recognisable as mammalian. Apart from these general resemblances the sperms of vertebrates are so varied that it is probable that any species could be identified by its sperm alone. Yet these variations appear to be of comparatively little functional importance and do not appear to have played any important part in the evolution of vertebrates.

CHAPTER III

OOGENESIS AND FERTILISATION

Oogenesis.—The stages in the formation of the ova are strictly comparable to those of spermatogenesis. The oogonia, which closely resemble the spermatogonia, divide an indefinite number of times and finally produce oocytes. The oocytes grow and undergo the heterotypic division. The two resulting cells are, however, unequal in size, one, the first polar-body, being very small and the other large. The large secondary oocyte then undergoes the homotypic division and produces the large ovum and the minute second polar-body. The first polar-body in many cases undergoes a similar division into two, each of the three resulting polar-bodies being homologous with the ovum. The unequal division of the cytoplasm at maturation thus provides for a rich supply of yolk being retained in the ovum at the expense of the polar-bodies, which degenerate.

Another difference between the male and female is to be found in the fact that the majority, if not all, of the oocytes in many vertebrates are formed and have completed the synaptic stages before puberty. They then enter a resting stage until the time for their liberation is near, when they undergo a rapid growth period which ends in the completion of the heterotypic division. The homotypic division then follows, usually without a pause. Sometimes the spermatozoa enter the egg before the maturation divisions have been completed, but nuclear fusion is not effected until after the formation of the second polar body.

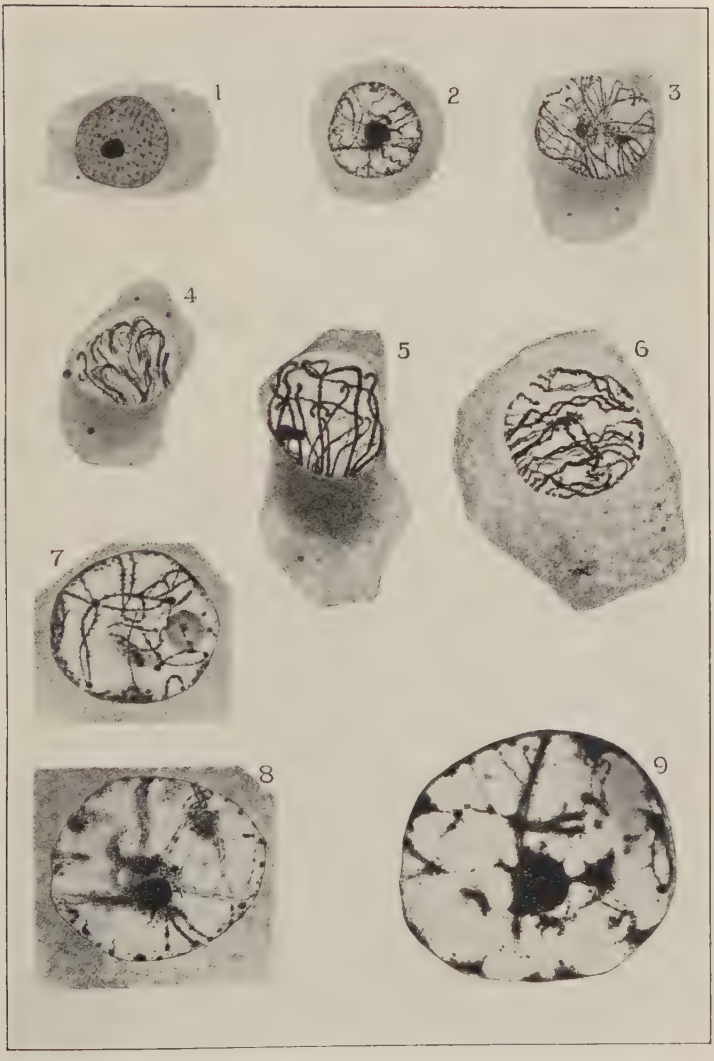
The cytoplasmic processes during oogenesis are characterised by the formation of the egg-membrane, or zona radiata, and large quantities of yolk. They are thus essentially different from those occurring in spermatogenesis. The

PLATE V

Nuclear Stages during Oogenesis in the Cat

1. Poussieroid stage. 2. Transition stage from deutobroque to leptotene. 3. Zygotene stages with the chromosomes oriented and beginning to pair. 4. Synizesis advanced. The chromosomes can be seen to be paired. 5. Pachytene nucleus. Syndesis complete. Synizesis nearly over. 6. Diplotene stage. 7. Transition stage from diplotene to dictyate. 8. and 9. Successive stages of dictyate nuclei in larger oocytes. × 1,150 diameters. (From de Winiwarter and Sainmont[236].)

PLATE V



nuclear changes, on the other hand, do not differ essentially, but rather in minor points. A brief account of de Winiwarter and Sainmont's [236] work on the oogenesis of the cat (Pl. V) will serve to show the essential similarity of the prophases with those described in the spermatogenesis of Lepidosiren.

The Nucleus in Oogenesis.—The diploid number of chromosomes in the cat appears to be thirty-six. The oogonial nuclei are oval and contain a central nucleolus and scattered granules of chromatin of variable size. The nucleus of the oocyte, prior to the onset of the prophase changes of the heterotypic division, is easily distinguishable from that of the oogonium. It is round and the chromatin is evenly distributed throughout it in the form of a fine dust with scarcely any traces of a reticulum. A rounded plasmosome and an oblong karyosome are always present, sometimes separate, at others joined together. These nuclei are termed " poussieroid " on account of the characteristic distribution of the chromatin as dust. They give rise to a transition stage by the fusion of the chromatin granules into larger grains, which become arranged in chains on chromatic filaments which tend to radiate from the central nucleus. The further condensation of the chromatin results in the filaments becoming more defined. They retain the radial arrangement around the nucleolus and wind about in the rest of the nucleus, especially immediately under the nuclear membrane. The karyosome is frequently large at this, the deutobroque, stage. This stage transforms into the leptotene in which the filaments are arranged in a spireme. They cease to radiate from the nucleolus and gradually become polarised and arranged in parallel pairs. The arrangement of the filaments in paired loops, the ends of which are concentrated at one pole of the cell, is complete in the zygotene stage, when it is possible by counting to show that there are about thirty-six filaments or chromosomes constituting about eighteen pairs. Synizesis, or contraction, occurs at this stage in the cat, which is earlier than in Lepidosiren, in which it occurs in the strepsitene or diplotene stage. Syndesis then takes place, the two chromosomes of each pair fusing throughout their length. This process is completed as synizesis comes to an end, and the

loops come to occupy again the whole cavity of the nucleus and are not polarised. Thus the term " bouquet stage " is applicable to the leptotene and zygotene nuclei, not to the pachytene, as in Lepidosiren.

The plasmosome retains its characters throughout these stages and is large. The karyosome, however, behaves in a remarkable manner. It is involved in the contraction of the chromosomes and is always eccentric. At the same time it develops a longitudinal constriction and can be seen to consist of two halves, closely applied to each other, in zygotene and pachytene nuclei. There can be little doubt that this karyosome represents the two sex- or X-chromosomes, on account of the similarity of its behaviour to those chromosomes in other animals (cf. Chapter IV).

The single filaments of the pachytene nuclei split in the diplotene nuclei, thus separating once more the individual chromosomes of each pair, as in Lepidosiren. The halves of the bivalents, however, still remain in proximity and more or less parallel to each other. The bivalents then lose their sharp contours and their staining affinities decrease. They become irregularly arranged in the cell and the chromatin granules become grouped in clusters of variable size. The nucleolus increases in size and small vacuoles can be distinguished in it. The karyosome, like the chromosomes, loses its staining capacity and becomes indistinguishable, and the whole nucleus increases in size. This is the dictyate stage of de Winiwarter, commonly known as the germinal vesicle. The oocyte remains in this condition for a variable time and completes its growth. At the approach of ovulation the chromosomes recondense and the nuclear membrane breaks down. The chromosomes, in the form of bivalents, become arranged on the spindle and the heterotypic division is effected. The germinal vesicle stage constitutes the chief difference between the nuclear changes in oogenesis and spermatogenesis, yet a similar resting stage is sometimes found in the latter, though never so accentuated. Agar [1] noticed such a stage intercalated into diakinesis in some of the spermatocytes of Lepidosiren. Moreover, he found such spermatocytes actually transforming

into oocytes in one specimen. The true spermatocytes of Lepidosiren in the resting stage have the diakinetic chromosomes arranged as flattened plates beneath the nuclear membrane and connected together by numerous bridges. Intermediate stages between these and the oocytes exhibited not only an increase in size, but a decrease in the staining affinity of the chromosomes, and an accumulation of a flocculent substance in the nucleus. The oocytes found in this one testis measured as much as 200 μ in diameter. Their nuclei contained indistinct strands of chromatin obscured by the presence of the flocculent substance. They had, in fact, all the essential characters of normal oocytes, both in the structure of the nucleus and the cytoplasm. This interesting observation emphasises the similarity of the early stages of spermatogenesis and oogenesis and probably indicates the manner in which oocytes can be formed in Amphibian testes after injury, as described in Chapter XI.

The chromosomes in the germinal vesicle stage of many vertebrates develop lateral fibrillæ which give them a hairy or feathery appearance. These are retracted at the close of this stage when the chromosomes recondense and the nuclear membrane disappears. The heterotypic spindle forms at right angles to the surface of the egg, division takes place and the first polar body is extruded. The homotypic spindle forms immediately and the chromosomes become arranged upon it, without an intermediate resting stage. The completion of the homotypic division is, as a rule, effected after the entrance of the spermatozoon. The polar spindles are always eccentric in vertebrates and the polar bodies are minute, relative to the size of the ovum. The first polar body usually enters on the homotypic division at the same time as the ovum, but may degenerate before the division is completed.

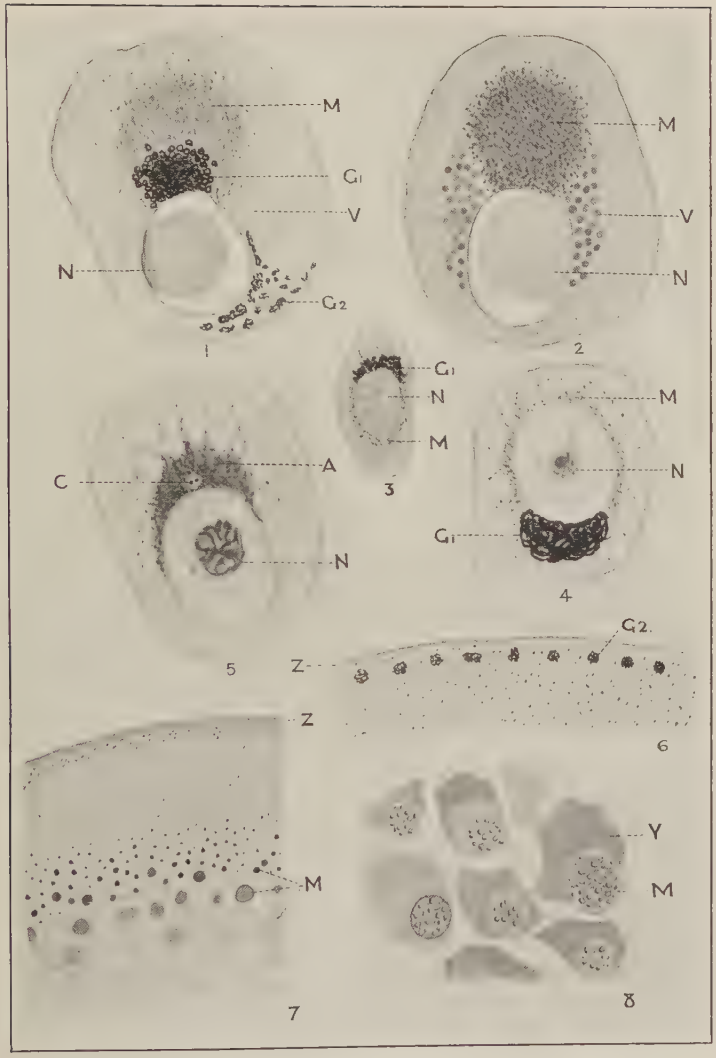
The Cytoplasm in Oogenesis.—The arrangement of the cytoplasmic structures in the young oocytes, prior to the onset of the growth stage, is essentially similar in all vertebrates. The subsequent stages, during which the yolk is being formed and the oocyte is growing, vary in detail in different species, but are probably similar in the more important points. Our knowledge

PLATE VI

Cytoplasmic Structures during Oogenesis in the Fowl

Oocyte ($\frac{1}{10}$ mm. in diam.) entered on the growth stage, showing the mitochondria (M) aggregated to form the yolk-sphere, the Golgi apparatus (G1) close to the nucleus (N), and the vacuoles (V) containing fatty yolk. Some Golgi bodies (G2) from the follicle cells have entered the cytoplasm of the oocyte. 2. Oocyte, similar to Fig. 1, showing the mitochondrial yolk sphere and the fatty yolk, but not the Golgi apparatus. 3. Oocyte before entering on the growth stage, showing the Golgi apparatus and the scattered mitochondria. 4. Oocyte at the outset of the growth stage. 5. Oocyte stained to show the archoplasm (A) from which the Golgi apparatus has been removed, and the centrosome (C) with two centrioles. 6. Portion of the periphery of a larger oocyte (1 mm. in diameter) showing Golgi bodies (G2) from the follicle cells, which have entered the cytoplasm, arranged in a row beneath the thin zona radiata (Z). 7. Portion of the periphery of an oocyte (2 mm. in diameter) showing mitochondria in a zone in various stages of swelling up to form mitochondrial yolk-spheres. 8. Portion of the yolk of a large oocyte showing the mitochondrial yolk-spheres (M) surrounded by yolk (Y) formed around them. (Modified from [27].)

PLATE VI



of the behaviour of the Golgi apparatus in the oocytes of vertebrates is unfortunately very meagre, but excellent accounts of the mitochondria in reptiles (Loyez [146], Bhattacharya [18]), birds (Loyez [146], Van Durme [222]), and mammals (Van der Stricht [219–221]) are available. The fowl provides a convenient type (Pl. VI), since it exhibits the process of yolk-formation in its most specialised form ([27], Van Durme [222]).

The young oocyte about to enter on the growth stage has the nucleus eccentric. The archoplasm, with the Golgi bodies studded over its surface, lies close to the nuclear membrane on the side of the nucleus farthest from the cell wall. It surrounds the centrosome, which consists of a small, clearly defined vacuole surrounding a pair of centrioles. The mitochondria are grouped around the archoplasm and close to the nuclear membrane in a crescentic area, the *croissant vitellogène*. As soon as growth starts the mitochondria become still more densely compacted and give rise to a solid mitochondrial sphere, or yolk-nucleus, close to both the archoplasmic sphere and the nuclear membrane. This mitochondrial yolk-nucleus is transitory in nature and soon breaks up again into a cloud of mitochondria which become scattered throughout the cytoplasm of the cell. Simultaneously the Golgi bodies and archoplasm break up into small fragments and also become dispersed throughout the cytoplasm. It is impossible to follow the centrosome after this has taken place. Fat-droplets appear in the cytoplasm while the dispersion of the mitochondria and Golgi elements is taking place, and mark, even at this early stage, the future vegetative or deutoplasmic pole of the oocyte. These fat-droplets do not arise from a direct transformation of either the mitochondria or Golgi bodies, although it is probable that these latter structures have an indirect influence on their formation. They become arranged in two zones, an outer and an inner, close to the nucleus. At this stage the follicle cells are supplying nutritive material to the periphery of the oocyte and are actually extruding portions of their own Golgi bodies into the periphery. The subsequent history of these Golgi bodies, derived from the follicle cells, and of those belonging properly to the oocyte, is uncertain, but it is probable that the

former disintegrate while the latter break up into fine granules which persist but do not appear to play a direct part in the elaboration of yolk.

The mitochondria, especially in a zone close to the periphery and another around the nucleus, give rise to the yolk spheres by direct transformation. The individual mitochondria swell up to many times their original size and develop vacuoles around them in which more yolk of a different kind is laid down. It is clear, then, that the deutoplasm of the vertebrate ovum is not made up of a single kind of yolk, but of several, and that the mitochondria are particularly concerned in its elaboration. The details are too complicated to describe here, sufficient having been given to outline the process. It is probable that the main features of yolk formation are similar in other vertebrates, but some of the phenomena, such as the formation of a definite mitochondrial yolk-nucleus and the extrusion of Golgi bodies from the follicle cells into the oocyte, are probably peculiar to those forms with macrolecithal ova. Indeed, the latter process is only known in birds and reptiles (Bhattacharya [18]). Pigment as well as yolk is developed in the oocytes of some vertebrates, such as the frog.

The Membranes.—The cytoplasm of the oocyte is covered by a very delicate vitelline membrane which closely adheres to it until fertilisation is effected. Outside this vitelline membrane is another thick membrane which is traversed by fine radial canals through which prolongations of the follicle cells communicate with the cytoplasm of the oocyte. These canals impart a radially striated appearance to the membrane from which it has derived its name of zona radiata. The zona radiata may consist of one or two layers, and may bear processes upon it, as in Teleosts, which serve to attach the egg when laid to the substratum. The zona radiata is very well developed in mammals, but in the full-grown oocytes of the Sauropsida it is usually thin, owing to having been stretched by the rapid growth.

The vitelline membrane is undoubtedly a true cell membrane and an integral part of the oocyte to which it belongs. It is, as such, the product of the cortical cytoplasm or ectoplasm

of the oocyte. The true nature of the zona radiata is not so clear, and some workers maintain that it is formed by the oocyte and others by the follicle cells. The latter view appears the more probable so far as the vertebrates are concerned. O. Van der Stricht [220, 221] and Thing [217] are of opinion that the zona radiata is laid down as a meshwork of terminal bars surrounding and separating the ends of the follicle cells, and arising from an intercellular substance or cement. This meshwork is then added to by the activity of the follicle cells. This view postulates the entirely follicular origin of the zona radiata.

Types of Vertebrate Ova.—The full-grown eggs of vertebrates vary enormously in size, composition, structure, etc. The smallest are those of Amphioxus and of Marsupial and Eutherian mammals. The ripe egg of Amphioxus only measures 0·1 mm. in diameter, that of the mouse 0·06 mm. in diameter, and that of man 0·2 mm. in diameter. These small or microlecithal eggs exhibit polarity, the nucleus being nearer one pole than the other. The deutoplasm is scattered throughout the cytoplasm, but is most plentiful in the hemisphere opposite the pole at which the nucleus is situated. The eggs of all other vertebrates contain much more yolk than these and are said to be macrolecithal. Among the Cyclostomes those of Petromyzon are fairly small, approximately 1 mm. in diameter, while those of Myxine and Bdellostoma are very much larger and are oval in shape. Teleosts also exhibit considerable variation in the size of their eggs, those of the ling, for instance, measure 1 mm. in diameter, while those of the salmon attain a diameter of 6·5 mm. The Cyclostomes and Teleosts exhibit great variation in the arrangement of the egg membranes, which are frequently provided with either horny hooks or processes, or with mucous envelopes, which serve as attachment organs. The Amphibia do not produce large eggs as a rule, those of the frog which measure about 1·6 mm. in diameter being typical. Some of the Gymnophiona, however, produce large oval eggs, as much as 9 mm. long, which are rich in yolk.

The eggs of the Elasmobranchs, Reptiles, Birds, and Monotremes (Fig. 2) are very large and rich in yolk. The ovum

(or yolk) of the hen being 40 mm. in diameter, while that of the common dog-fish is about the same size. The ovum of the ostrich measures about 80 mm. in diameter, while Wilson [230] found that the eggs of a large shark (*Chlamydoselache*) exceeded this size. Wilson estimates, by comparing the shell with those of other birds, that the ovum of the extinct bird of Madagascar, Æpyornis, must have been about 175 mm. in diameter. The ova of Monotremes measure 3 to 4·75 mm. in diameter.

These yolk-laden eggs have the germinal vesicle situated immediately beneath the vitelline membrane at one pole of the egg. It is surrounded by a disc of protoplasm, almost free from yolk, the germinal disc, which is continued into the centre of the egg in the form of a well or " latebra " of protoplasm, containing fine yolk-granules. The remainder of the cell is filled with larger yolk-granules which may exhibit a concentric or stratified arrangement.

The number of ova produced at a time is roughly inversely proportional to their size. Thus Amphioxus liberates enormous numbers at each laying, as do many of the Teleosts. A 54-lb. ling (Cunningham [61]) has been shown to produce twenty-eight millions at a time. The Elasmobranchs, Sauropsida, and Monotremata, on the other hand, only produce very few eggs at a time, usually only one or two. The Marsupial and Eutherian mammals are an exception to this rule, since, despite the small size of their ova, they produce them in very small numbers ; this is correlated, of course, with the advantages of internal fertilisation and embryonic development.

These facts show that the primitive ancestral vertebrate produced large numbers of small eggs. The general trend of vertebrate evolution has been in the direction of reducing the number and enlarging the size of the eggs produced. This modification reaches its climax in the Sauropsida, but ends there, since, with the evolution of the gestation habit and of the placenta, it was not advantageous to the Mammalia. Consequently their ova have undergone a secondary reduction in size, slight in the Monotremes, in which gestation is short and there is no placenta, but very marked in other forms. This simple explanation is of fundamental importance, since on it

depends the evolution of the sexual characters in the vertebrates, and more particularly the remarkable modifications of the reproductive system which characterise the Mammalia.

Fertilisation. - The sperm, in many vertebrates, enters the ovum after the separation of the first polar body. The second maturation spindle has formed and proceeded as far as the metaphase in some forms, while in others it has reached the anaphase before the sperm enters. The completion of the formation of the second polar body in these forms appears to depend on the entrance of the sperm.

More than one sperm normally enters the ovum in many vertebrates, including Elasmobranchs, Reptiles, Birds (see Wilson [230]), Monotremes (Hill and Gatenby [111]) and many Amphibia. Polyspermy thus appears to be common, though not universal, in the forms with macrolecithal eggs. Only one of the sperms actually effects fertilisation, and its nucleus fuses with the pro-nucleus of the ovum. The supernumerary sperms degenerate early in some forms, notably the Urodeles, while in others they form vesicular pro-nuclei and may even proceed to divide, before degenerating.

Among mammals, other than Monotremes, only one sperm normally enters the egg. The actual process of fertilisation has been worked out in detail in the bat by O. Van der Stricht [220] and in the guinea-pig by his pupil Lams [136]. The description by the latter author will be taken as an example (Pl. VII), since the structure of the guinea-pig sperm has been dealt with already in some detail.

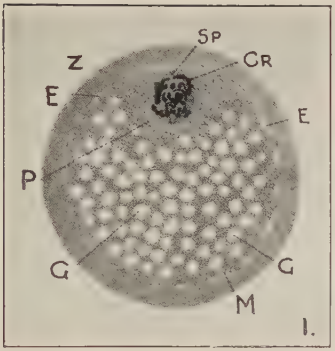
The full-grown oocyte of the guinea-pig contains an eccentric nucleus in the germinal vesicle stage. The mitochondria are scattered throughout the cytoplasm and numerous fat spherules are present in a crescentic area around the nucleus. The oocyte at this stage has attained its maximum size of about 80 μ in diameter. The nuclear membrane then breaks down, and the chromosomes, which have recondensed, are set free in the cytoplasm. The chromosomes arrange themselves on the spindle, which is peripheral and roughly perpendicular to the surface of the oocyte. The heterotypic division follows and the first polar-body is formed. The second maturation spindle

PLATE VII

Fertilisation Stages in the Guinea-pig (Cavia)

1. Spindle of first maturation division. 2. Second maturation division just completed. The head of the spermatozoon in the cytoplasm is beginning to swell. 3. The head of the sperm, with the tail still attached, is swollen to form the male pronucleus. It is surrounded by a large aster. The female pronucleus is formed, but is still small. It is surrounded by a small aster. 4. The two pronuclei are fully formed and the chromosomes can be distinguished in them. The tail of the sperm is still close to the male pronucleus and can be seen to consist of two filaments. The male pronucleus is, characteristically, the larger and more peripheral. 5. First segmentation spindle. 6. Two blastomere stages. The two polar bodies and the detached masses of deutoplasm can be distinguished. The tail of the spermatozoon is still visible in one blastomere. ×510. (From Lams [136].)

Guide letters :—A, aster ; Cr., chromosomes ; D, deutoplasmic pole; Dl., products of deutoplasmolysis ; E, peri-vitelline space ; Sp., spindle ; G, fat-droplets (Q in Fig. 5.) : Pb., polar bodies (first and second) ; M, vitelline membrane ; N, nucleus ; P, plastic pole ; Q, tail of spermatozoon ; S, spermatozoon ; T, head of spermatozoon ; Z, zona radiata ; ♀, female pronucleus ; ♂, male pronucleus.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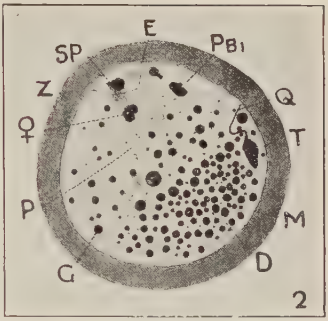
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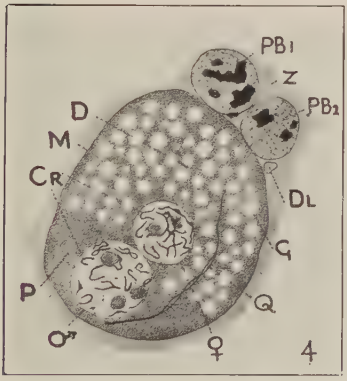
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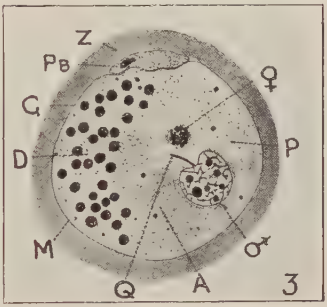
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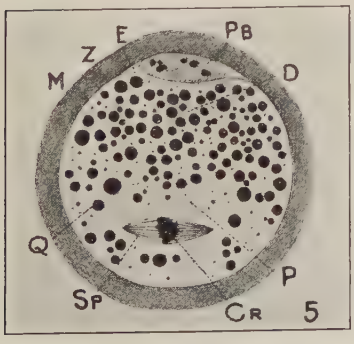
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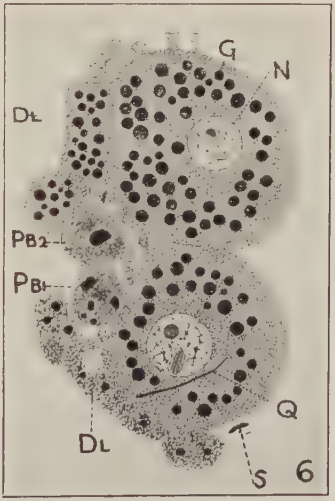
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forms at once, without the intervention of a resting stage, and the chromosomes become arranged in the equatorial plane. The second maturation spindle is formed in a projecting lobe of the oocyte, close to the first polar body, and is parallel or oblique to the surface. Ovulation takes place at this stage and the oocyte passes into the tube. The completion of the homotypic division and the separation of the second polar body is dependent on the entrance of the spermatozoon. Should this not take place the oocyte degenerates. Fertilisation is effected while the oocyte is in the Fallopian tube, the sperm penetrating the zona radiata and entering the substance of the oocyte. The entire sperm enters the oocyte head-first. Immediately after the entrance of the sperm the homotypic division is completed and the second polar body is formed.

The polar bodies, after their separation, persist as degenerate bodies flattened between the ovum and the zona radiata. The first polar body of the guinea-pig, unlike that of many other vertebrates, does not undergo a homotypic division corresponding to that of the ovum. After the separation of the polar bodies the ovum becomes detached from the surrounding membranes and separated from them by a narrow fluid-filled, peri-vitelline space. This space appears to prevent the entrance of supernumerary sperms and allows of the free orientation of the ovum. The ovum eliminates much of its deutoplasm into this space. The elimination is effected by the concentration of the deutoplasm into lobes on the periphery of the ovum which finally become separated from it. This process, called deutoplasmolysis, begins after the formation of the second polar body and may continue during the early stages of segmentation.

The centrosome and achromatic filaments of the ovum disappear immediately after the separation of the second polar body and the group of chromosomes become surrounded by a nuclear membrane and form the female pro-nucleus. This pro-nucleus, at first very small, rapidly swells and attains its full size. The chromatin becomes arranged on an achromatic reticulum and several nucleoli become apparent. The pro-nucleus at the same time migrates towards the pole of the ovum

away from that at which the polar bodies were formed. This results in a displacement of the fat-globules to the opposite pole.

Meanwhile the head of the sperm has been undergoing similar changes, and has swollen to form the male pro-nucleus, which is similar but slightly larger than the female. It migrates also to the animal pole, if it is not already there. Both pro-nuclei then take up an eccentric position close together, the male being peripheral and the female between it and the polar bodies. A line joining their centres therefore marks the axis of the egg and is in the plane of the first cleavage. The pro-nuclei do not fuse, but enter separately on the prophase changes of the first cleavage. The chromosomes condense and are set free in the cytoplasm by the dissolution of the nuclear membranes. The spindle with asters forms at right angles to the axis of the egg, the chromosomes become arranged on its equator, and cleavage follows.

The fate of the middle-piece and tail of the sperm meanwhile is more difficult to follow. They enter the ovum with the head and remain attached to it during its migration and transformation into the male pro-nucleus. Sometimes the axial filament can be seen to separate partially into two fibrillæ. The proximal centrosome of the sperm separates from the axial filament and forms an aster which extends throughout the entire ovum, but apparently disappears before the formation of the first cleavage spindle. This centrosome probably takes part in the formation of this spindle, and may be, as in many animals, solely responsible for it. During the first cleavage the tail of the sperm passes into one of the blastomeres and has not been traced subsequently in the guinea-pig.

The essential phases of this process are the same in all vertebrates, but the details vary. The sperm does not perforate the zona radiata, but enters the ovum by a micropyle in Mixinoids and many Teleosts. It perforates the zona radiata in other forms. It enters in the upper hemisphere of the ovum in Amphibians, and in the germinal disc in Elasmobranchs, Sauropsida, and probably in Monotremes. The sperm may enter any part of the surface of the ovum in other mammals, as in Cavia. The ovum sometimes forms an attraction cone at

the point of contact with the sperm, which facilitates its entry. The formation immediately after the entry of the sperm of a fluid-filled peri-vitelline space appears to be general. Deutoplasmolysis, on the other hand, is characteristic of the mammals other than Monotremes. It was first observed in the bat by O. Van der Stricht [219] and has since been noted in a number of other forms including the mouse, cat, dog, Dasyurus and Didelphys. Dasyurus, described by Hill [110], is the most extreme case. The major part of the deutoplasm is eliminated in the form of a single large globule, before the first cleavage, leaving the ovum practically free from deutoplasm. Deutoplasmolysis, as Hill and Tribe [113] have pointed out, is to be interpreted in the light of the phylogeny of the Mammalia. In other words, it is an adaptation of the ova, already much reduced in size compared with those of the Sauropsida, to reduce their bulk still further so as to facilitate holoblastic cleavage.

It is unfortunate that little is known of the function or ultimate fate of the cytoplasmic constituents of the sperm, other than the proximal centrosome, which are introduced into the cytoplasm of the ovum at fertilisation. The acrosome appears to break up and disappear soon after it has entered the ovum, and the only function that can be ascribed to it at present is that it may effect the perforation of the egg membranes or the attachment of the sperm to them. Virtually nothing is known of the fate in vertebrates of the sperm mitochondria and Golgi bodies contained in the middle-piece. Yet these various bodies constitute the cytoplasm received from the male parent and are equivalent, from the point of view of cytoplasmic inheritance, to the entire cytoplasm of the egg. It is highly desirable that these structures should be followed, by means of modern technique, through the stages of fertilisation and segmentation.

CHAPTER IV

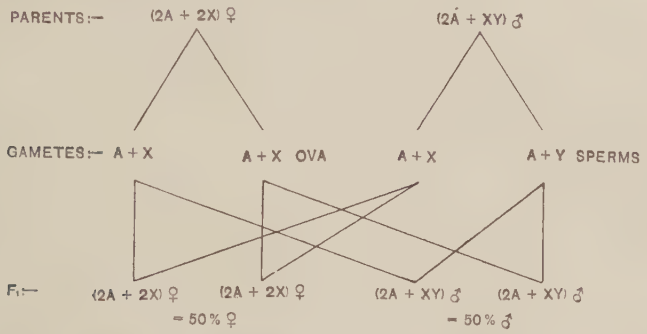
THE SEX CHROMOSOMES

General Aspects.—The chromosome theory of sex-determination in its general form may be simply stated. The number of chromosomes in one sex is even and consists of homologous pairs. One of these pairs consists of two equivalent sex chromosomes which often can be distinguished cytologically. The other chromosomes, which are similar in both sexes, are known as the autosomes. The reduction division results in the separation of one member of each pair to each daughter-cell. Therefore all the gametes produced have the same chromosomal constitution. This sex is said to be homogametic.

The opposite sex differs in that one sex-chromosome is absent or is represented by an unequal chromosome. In the latter case the two sex-chromosomes are dissimilar and the reduction division results in one going to each daughter-cell. In the former case the sex-chromosome behaves in a similar manner and is distributed to half the daughter-cells. These two conditions are essentially similar and result in the production of two different kinds of gametes in equal numbers. Half the gametes have the same chromosomal constitution as those of the other sex, and half lack the sex-chromosome or contain the unequal partner. This sex is therefore said to be digametic or heterogametic. It can be seen at a glance that this arrangement will result in half the fertilised ova having the chromosomal constitution of one sex and half that of the other sex.

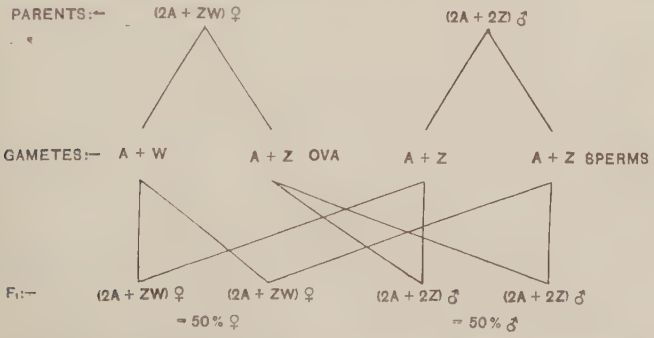
The male is the heterogametic sex in the majority of animals. When this is the case the sex-chromosome common to both sexes is called the X-chromosome, and its unequal partner in the male is known as the Y-chromosome. The chromosomal

distribution in the parents, gametes, and offspring is represented diagrammatically in the following figure, where A=a haploid set of autosomes.



This scheme is applicable to the condition in which the Y-chromosome is absent if O is substituted for Y.

The female is the heterogametic sex in birds, in moths and butterflies, and probably in some fish and amphibians. It is customary to represent the sex-chromosome in them by Z instead of X, and its partner in the female by W. This is shown in the following scheme :



The sex-chromosome appears to have been observed by a number of workers during the last twenty years of the nineteenth century, but its nature and significance were not understood. The sex-chromosome theory as such was formulated by McClung [147] in 1901. Since then an immense body of evidence has been accumulated in support of it.

This evidence has been derived from two sources; from a cytological study of the chromosomes, and from genetical studies of certain hereditary characters associated with sex.

We will consider first the cytology of the sex-chromosome, our knowledge of which has been admirably reviewed by Wilson,[230] a pioneer in this field of research, and by Schrader[204].

The sex-chromosomes in the heterogametic sex are recognisable as a rule by their peculiar behaviour in comparison with the autosomes.

The most important characteristics are a tendency to undergo precocious condensation (*heteropycnosis*) in the growth stages and to pass to the poles of the meiotic spindle before (*precession*) or after (*succession*) the autosomes. In some forms the heterotypic division of the sex-chromosomes takes place at the second maturation division instead of at the first, which is reductional for the autosomes. The absence of a similar partner renders syndesis impossible and results in the failure of the sex-chromosomes to become elongated and polarised like the autosomes in the bouquet stages. The sex-chromosomes are sometimes included in special chromosome vesicles or may be associated with plasmosomes. None of these characters alone is sufficient to distinguish the sex-chromosome, as they are occasionally exhibited, under certain conditions, by autosomes. The behaviour of the sex-chromosomes in the heterogametic sex differs from that of the autosomes in degree and chronology, rather than in kind, as Wenrich[227] has pointed out. The peculiarities commonly associated with them are probably largely due to the lack of a similar partner. This view is strongly supported by the fact that in the homogametic sex, where they form an homologous pair, their behaviour is similar to that of the autosomes, from which they can be distinguished only in certain cases by peculiarities in their shape and size.

A remarkable phenomenon, associated with the sex-chromosomes, is the absence or reduced amount of crossing over which takes place in the heterogametic sex as compared with the homogametic. Linkage of the genes in all the chromosomes

is, as it were, tightened in the heterogametic sex. Haldane [100] and Huxley [120] have suggested that this is an adaptation to prevent crossing over between the X- and Y-chromosomes which would lead to many complications. They suggest that heteropycnosis provides another means of preventing crossing over between the X- and Y-chromosomes in the heterogametic sex without necessarily interfering with linkage in the autosomes.

Cytology.—The simple condition of the sex-chromosomes is sometimes complicated by the division of the X- or Y-chromosome into several parts (*compound sex-chromosomes*) or by the adherence of sex-chromosomes to autosomes (*multiple chromosomes*).

The compound Y is much more rare than the compound X. The compound X-chromosome is usually accompanied by a single Y, but in some cases it has no partner. The components of compound chromosomes, whether sex-chromosomes or autosomes, are, however, not entirely independent, as is shown by the fact that they all segregate to one daughter-cell at meiosis. It seems clear that, in most cases, such compound chromosomes have originated by the breaking up of a single chromosome.

Multiple chromosomes in any one individual or species involve certain definite members of the chromosome group. When a sex-chromosome is attached to an autosome in the heterogametic sex, the multiple nature is revealed at once in the maturation stages by the heteropycnosis of the former, while the latter forms a tetrad with its free partner and divides in a normal manner.

Supernumerary or extra chromosomes are occasionally found and are liable to be confused with sex-chromosomes. They usually exhibit heteropycnosis, but Carroll [49] has shown that when a pair are present they undergo no heteropycnosis. Schrader [204] considers that supernumeraries may, in some instances, originate from sex-chromosomes. They have, however, apparently no influence on sex-determination.

The head of the mature spermatozoon is constituted almost entirely of the nucleus and acrosome, as we have already

shown. Further, the nucleus is composed of a compact mass of chromatin. It might be expected, therefore, that the presence or absence of the X-chromosome in sperms could be detected by measurements of the heads. Such a dimegaly has been demonstrated, by a number of workers, in the spermatozoa of many animals, both vertebrate and invertebrate. Among the mammals, especially, much work has been done, giving positive results in man, pig, horse, bull, dog, rat, and mouse.

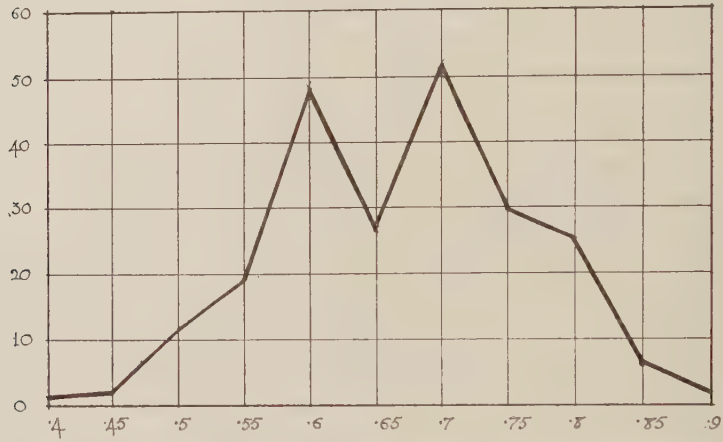


FIG. 3.—Frequency curve of head lengths of human spermatozoa. The bimodal nature of the curve indicates dimorphism of the spermatozoa. (From Parkes[170].)

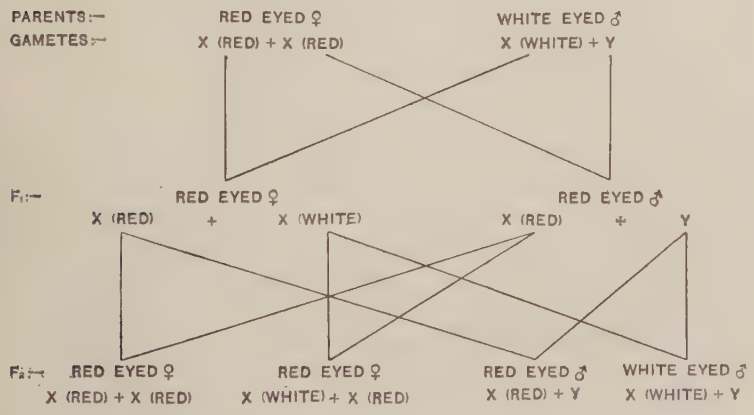
Parkes [170] plotted his measurements of human spermatozoa as a frequency distribution, and obtained a bimodal curve as shown in Fig. 3.

This evidence appears to support the sex-chromosome theory, but it must not be stressed, because simple calculations will show that the size differences between the two kinds of spermatozoa, shown by this means, are frequently far greater than can be accounted for by the mere presence or absence of an X-chromosome.

Genetics.—Breeding experiments furnish the second line of evidence in support of the sex-chromosome theory. These experiments are based on the simple theoretical expectation following from the assumption that the X-chromosome bears,

as well as those that determine sex, other hereditary factors or genes. Should this be so, the characters invovled will be inherited by the heterogametic offspring from the homogametic parent alone. Many characters in a variety of animals have been observed to conform to this theoretical expectation and are said to be *sex-linked*.

A simple example of sex-linked inheritance is furnished by the fruit-fly, Drosophila. Two X-chromosomes have been demonstrated cytologically in the female and a single X accompanied by a Y in the male. The X-chromosome may carry either the gene for red eyes or, alternatively, that for white eyes, but not both. Such alternative genes are called allelomorphs. A fly which is heterozygous for this character, or has both genes, will have red eyes. Therefore the gene for red eyes is said to be dominant. The gene for white eyes, which is not expressed in the presence of its dominant partner, is said to be recessive. A female which contains either gene in duplicate is said to be pure or homozygous for that character. It is obvious that the male fly, having only one X-chromosome, must be, as a rule, pure for any sex-linked character, since the Y-chromosome in most forms bears no genes or only abortive ones. When a male with white eyes is mated with a female pure for red eyes all the offspring have red eyes.

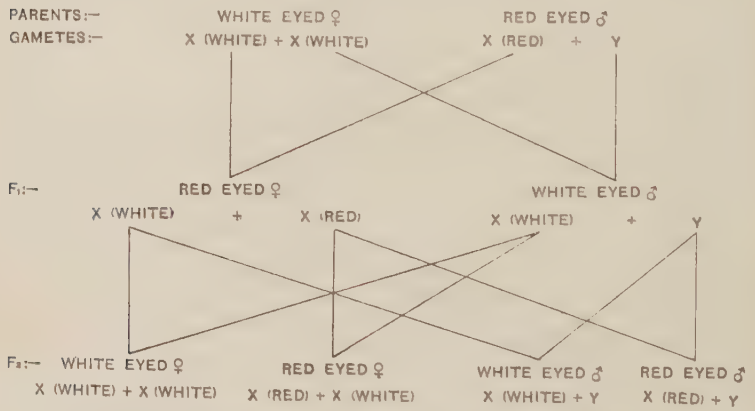


The diagram shows that when these are mated together all

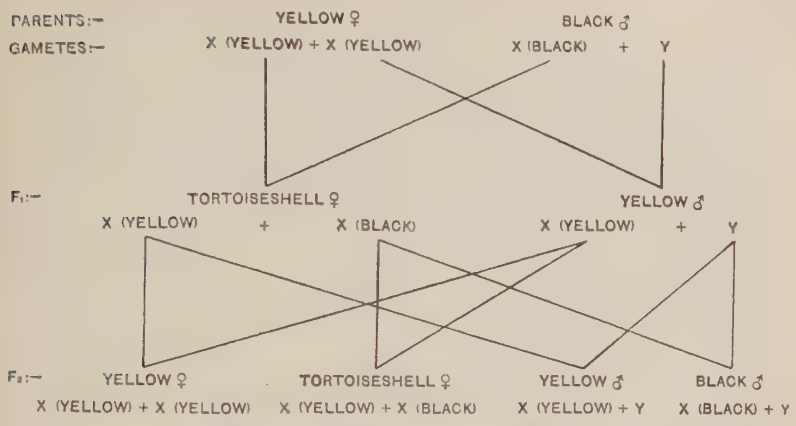
the females and half the males of the resulting, or F2, generation have red eyes, and the rest of the males have white eyes.

In this and the subsequent diagrams the full name of the character is given in brackets beside the chromosome which bears its gene. This method has been employed rather than the usual Mendelian symbols of a capital letter for the dominant gene and the corresponding letter for its recessive allelomorph, for the sake of simplicity.

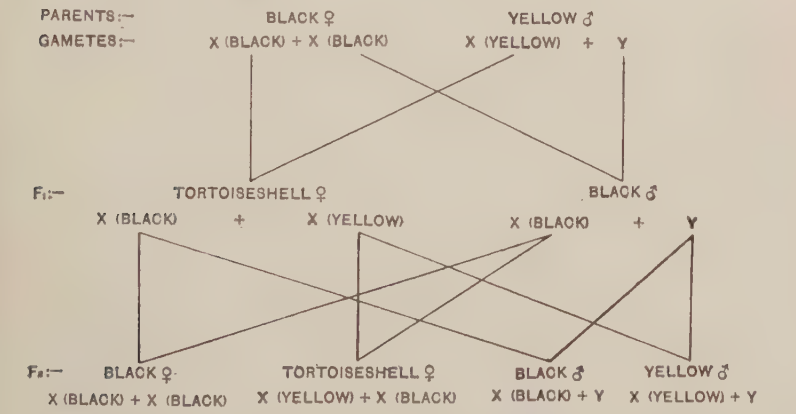
The reciprocal cross of a white-eyed female with a red-eyed male results in criss-cross inheritance in which all the males of the F1 generation have white eyes and all the females red eyes. When these are mated together 50 per cent. of the males and 50 per cent. of the females of the F2 generation have red eyes, and the rest have white eyes.



Among the vertebrates this form of sex-linked inheritance is not uncommon in mammals. A good example is furnished by the tortoiseshell cat, where the genes for black coat and yellow coat are sex-linked allelomorphs. These are not dominant and recessive to each other, but constitute, when both are present, the tortoiseshell character. Therefore only heterozygous females are tortoiseshell, the males and pure females being yellow or black as the case may be. The cross between a yellow female and a black male is shown achematically as follows :

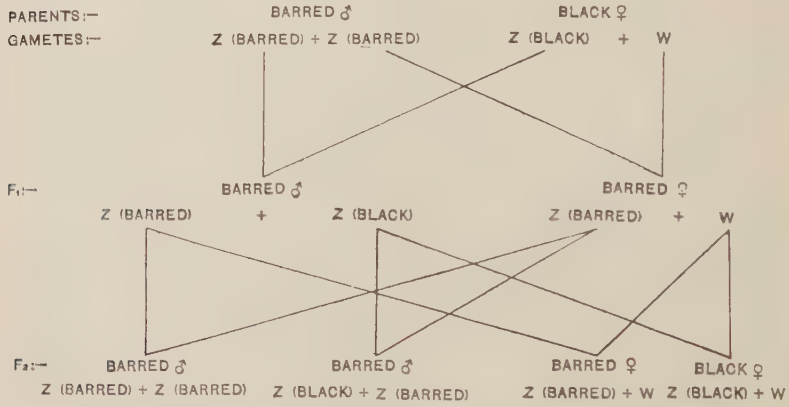


Black females are occasionally produced in the F2 generation of this cross and the F1 generation of the reciprocal cross. The origin of these is uncertain, but they may be heterozygotes in which the black alone has been expressed. Tortoiseshell males also appear exceptionally and are usually sterile. The reason for their appearance is still obscure. These rare exceptions do not interfere seriously with the interpretation given, which is generally accepted.

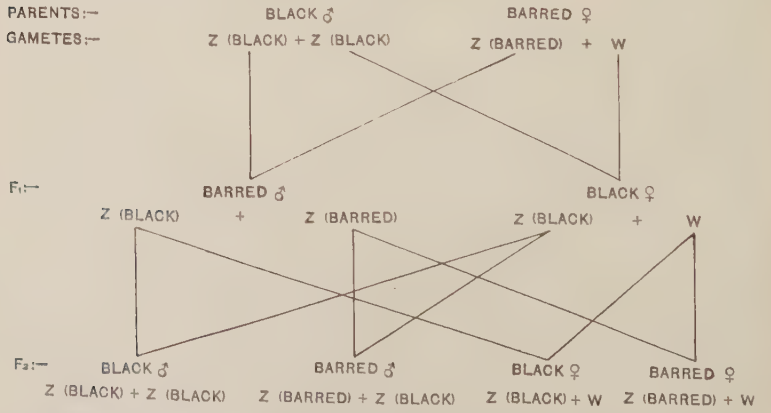


Sex-linked characters behave in a similar manner in birds and moths, except that in them the daughters being heterogametic derive their sex-linked characters from the male

parent. Many plumage characters in fowl are sex-linked, as, for instance, barred and non-barred, gold and silver, etc. Barred and silver are dominant, non-barred and gold recessive. If a Plymouth Rock male is mated with a Langsham female all the F1 generation are barred like the Plymouth Rock. When these are interbred all the males and half the females of the F2 generation are barred, and half the females are black like the Langsham.



In the reciprocal cross all the sons of the F1 generation are barred and all the daughters black.



When these are interbred half the sons and half the daughters of the F2 generation are barred and half are black.

This sex-linkage of plumage characters provides an easy means of determining sex with certainty in the F1 generation at hatching. The newly hatched females in the F1 generation of the last-mentioned cross are pure black, whereas the males are black with a white spot on the top of the head and white underneath and are thus easily distinguishable even in the downy condition.

Occurrence of Sex-chromosomes in Vertebrates.—Sex-chromosomes have been described in a large number of forms both vertebrate and invertebrate. Among the invertebrates they have been recognised in some Platyhelminthes, Nemathelminthes, Mollusca, Arthropoda, and Echinodermata. Among the chordates they have been found in members of all the vertebrate classes. In a considerable number of cases, especially among the insects and vertebrates, their existence has been inferred also from sex-linked inheritance. They have not been found among the Protochordates nor the other invertebrate phyla. Our knowledge of the cytological and genetical behaviour of the sex-chromosomes is most extensive in the insects among invertebrates, especially in the fruit-fly Drosophila.

The cytological evidence of the occurrence of sex-chromosomes in fishes is extremely flimsy. The genetical evidence, however, shows that the males in several species of Teleosts are heterogametic (for references, see Schrader [204]). This conclusion is supported by the work of Huxley [117] on sex-reversal in the Millions-fish, to be referred to later. On the other hand, the work of Bellamy [13] and Gordon [96] on Platypœcilus indicates that the females are heterogametic.

The conditions among the Amphibia are far from clear, although a large amount of work has been done on them. Among the Urodeles, King [128] has described a single X-chromosome, attached to an autosome, and possibly a Y, in the male Necturus. More recently Witschi [244] has described an X–Y pair in the male of Rana temporaria. The cytological evidence, while not conclusive, indicates that the male is heterogametic. Cases of sex-reversal in frogs, to be described

in Chapter XI, provide genetical results which amply confirm this indication.

The chromosomes of the reptiles (Fig. 6) furnish difficult material for exact cytological study as they consist of a number of very small chromosomes or microchromosomes and a few large macrochromosomes. Fortunately the sex-chromosomes are among the latter, thus enabling several workers (for references, see Schrader [204]) to show that the male is heterogametic. The

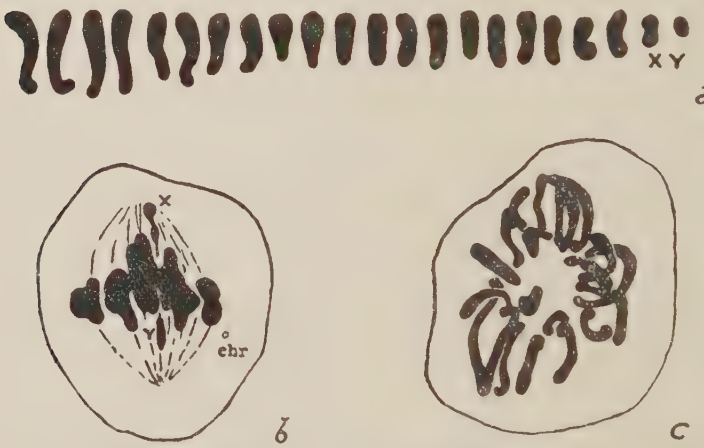


FIG. 4.—The chromosomes of the opossum (Didelphys Virginiana). (*a*) Spermatogonial chromosomes arranged approximately in the order of their size. The X- and Y-chromosomes are labelled. (*b*) Spermatocyte showing the X- and Y-chromosomes separating on the first maturation spindle. (*c*) Equatorial plate of a dividing somatic cell of a male embryo. (From Painter [169].)

X-chromosome appears to be simple in some lizards and compound in others and with or without a Y.

Our knowledge of the sex-chromosomes in birds is virtually confined to the fowl and the pigeon. The chromosomes of these closely resemble those of the reptiles in consisting of a few macrosomes, which include the sex-chromosomes, and a number of microsomes. Several workers are agreed that the male in the fowl is homogametic and the female heterogametic (Fig. 5). The Z-chromosomes are the largest of the macrosomes, and that in the female is accompanied by a small W. Oguma [165]

describes a similar condition in the pigeon, but could not distinguish a W. These findings are confirmed by the sex-linked inheritance of a large number of factors, mostly for

FIG. 5.—Chromosome plates in the somatic cells of fowl. That on the left is from a female and shows an X- (X) and a Y-chromosome (Y). That on the right is from a male and shows two X-chromosomes (X). (From Shiwago [207].)

plumage characters, in fowl, pigeons, canaries, etc., such as those already described in the Plymouth Rock–Langsham crosses.

Among mammals the cytological evidence in a very large number of forms clearly demonstrates that the male is the heterogametic sex; a finding which is in accordance with the genetical expectations. The conditions in Monotremes are not so far understood, but the Marsupials have been well worked

FIG. 6.—Chromosomes of a Lizard (Anolis). (*a*) Spermatogonial equatorial plate. (*b*) Primary spermatocyte showing tetrads. The X-chromosomes are labelled. (*c*) Spermatocyte showing the X-chromosome (X) passing undivided to one pole on the first maturation spindle. (From Painter [168].)

out. They furnish clearer results than the Eutheria on account of the relatively smaller number of chromosomes (Fig. 4). The X-chromosome in them is considerably smaller than the autosomes and is accompanied by a minute Y. Among the

Eutheria it is equally clear that the male is heterogametic, but there is considerable disagreement as to whether the condition in various forms is XO or XY. In man, de Winiwarter [235] and others consider that there is no Y-chromosome (Fig. 7), but Painter maintains that there is (for references, see Schrader [204]). The latter conclusion is borne out by the fact that the web-toed character in man appears to be transmitted through the male line only and is, therefore, presumably, borne by the Y-chromosome (Schofield [203]). In view of the conflicting

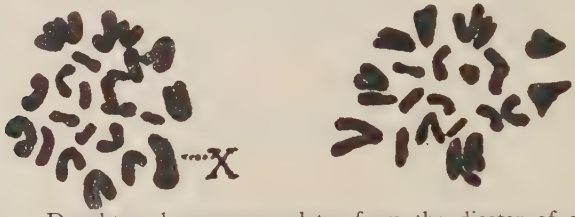


FIG. 7.—Daughter chromosome plates from the diaster of a human primary spermatocyte. That on the left contains one X-chromosome (X), while that on the right has none. (From de Winiwarter and Oguma [235].)

evidence it does not appear possible to arrive at a definite conclusion on this point at present.

Theoretical Considerations.—The occurrence of the sex-chromosomes in so many forms, always correlated with sex, and the genetical evidence of sex-linked inheritance afford ample proof of the sex-chromosome theory. Consequently the problems of the origin and evolution of the sex-chromosomes and of the manner in which they determine sex have been the subjects of much speculation. These speculations have been treated at some length by Wilson [230] and by Schrader [204], and no systematic attempt to deal with them is necessary here. A few of the more important points, however, may help in the consideration of the subsequent chapters.

The following remarks will be mainly confined, for the sake of simplicity, to the XX♀ XY♂ condition, which is more common than female heterogamity. Also our knowledge of the cytology and genetics of the sex-chromosome in Drosophila, in which the male is heterogametic, is much more complete than in the case of any other animal. The behaviour

of the sex-chromosomes in birds and Lepidoptera is so like that in other forms that it is probable that these considerations apply equally well to them, provided allowance is made for the female being the heterogametic sex.

Wilson has compiled a series of forms ranging from those in which no heteromorphic pair of chromosomes can be distinguished cytologically to those in which the Y-chromosome is absent altogether. Although this series is not phylogenetic, it suggests that the X and Y originally formed an homologous pair, capable of synapsis, and indistinguishable in appearance and behaviour ; a condition which has persisted in some forms which exhibit sex-linked inheritance. The gradual cessation of function accompanied by reduction in size of one member of such a pair in the heterogametic sex would result in the production of an X accompanied by a more or less inactive Y. Once this condition is achieved it is easy to imagine the ultimate complete disappearance of the Y resulting in the XO condition observed in many forms. This hypothesis is substantiated by our knowledge of the genetics of the Y-chromosome, which appears to be functionless in some forms and to carry a few genes in others. The occasional absence of the Y-chromosome in the male Metapodius, where one is usually present, produces no visible effect (Wilson [230]). Bridges [39] has shown, however, that the absence of the Y in the male Drosophila causes sterility. Moreover, in the fish, *Lebistes reticulatus*, a small black spot on the dorsal fin was transmitted through the male line only for five generations (Schmidt [202]), the gene for which was presumably carried by the Y-chromosome. The factor for the web-toed character in man is apparently borne also by the Y-chromosome (Schofield [203]).

These considerations lead on to the second problem : the manner in which the sex-chromosomes determine sex. The Y-chromosome appears to take no essential part in sex-determination for two reasons : (*a*) It is absent altogether in many forms, and (*b*) the accidental inclusion of an extra Y in the zygote of Drosophila does not affect the sex of the resulting fly.

The X-chromosomes are therefore normally the decisive

factors in determining sex. Their influence in this respect obviously must be either qualitative or quantitative. A little thought will show that the latter conception fits the facts. It is, for instance, the X-chromosome of the male which, when passed on to the offspring, determines that it shall be female. The quantitative action is shown further by a number of recorded cases in Drosophila in which the sex-chromosomes failed to separate in the maturation divisions. Owing to this non-disjunction of the sex-chromosomes, as it is called, germ-cells were produced containing two X's. A good example is that recorded by Bridges,[40, 41, 42] who obtained, mainly through a chance occurrence of triploid females, strains of Drosophila with one, two, three or four X-chromosomes variously combined with diploid, triploid and tetraploid sets of autosomes. Those individuals which had three X-chromosomes and three haploid sets of autosomes or four X-chromosomes and four haploid sets of autosomes were practically normal females, but those with two X-chromosomes and three haploid sets of autosomes were intersexes. The individuals with three X's and two haploid sets of autosomes and with one X and three haploid sets of autosomes were abnormal sterile females and abnormal males respectively.

The results can be summarised as follows : where A stands for a complete haploid set of autosomes.

2A+3X=Super female.
4A+4X=Female.
3A+3X= ,,
2A+2X= ,,
3A+2X=Intersex.
2A+1X=Male.
3A+1X=Super male.

These observations confirm the hypothesis that the effect of the X-chromosomes is quantitative. They show further that it is not the absolute quantity of X-chromatin which determines sex, but the quantity relative to the autosomes. The effect of the autosomes on sex is elucidated still more by the work of Sturtevant[212] on a certain stock of Drosophila,

which showed that the presence of a recessive gene, situated in the second chromosome (autosome), changed individuals with two X-chromosomes, which should therefore have been females, into intersexes of a definite type.

Although the X-chromosomes are usually the decisive factors in determining sex, the autosomes are involved also and possibly even the cytoplasm. Morgan [158] suggested the use of a symbolic method of representing sex in forms in which the male is heterogametic, which fits these facts remarkably well. He represented the factor for maleness by M, and assumed that it was borne by the autosomes and was present equally in all germ-cells. The factor for femaleness, F, was assumed to be present in all the X-chromosomes. All the ova will then carry the factors FM and the sperms will be either FM or M, according to whether they carry an X-chromosome or not. Consequently the fertilised egg will be either FFMM or FMM. The former will be female and the latter male on the further assumption that F>M but F<MM. It must be remembered, however, that this formula is only a rough, though convenient, symbolism and must not be pressed too far.

Goldschmidt [92] adapted this formula to make it applicable to the intersexes which appear in certain racial crosses of the Gipsy moth, *Lymantria dispar*. It is necessary to reverse the formula in this case, as the female is heterogametic, and the Z-chromosome presumably carries the factor M. Goldschmidt considered that the factor F was borne in the cytoplasm of the egg or more probably by the W-chromosome. He found that in certain crosses chromosomal females developed the male characters so completely that they became functional males. He was able to account roughly for the grade of intersexes appearing in any given cross by assigning arbitrary numerical values to these factors. These numerical values are, of course, purely symbolical.

The sex-chromosome mechanism appears to be the same in all essential respects in forms in which the heterogametic sex is the male and those in which it is the female. The relation of these two types presents an interesting problem at

present unsolved. There appears to be no sound reason for assuming one to be more primitive than the other. Male heterogamety is certainly the more common, but the existence of female heterogamety in Lepidoptera and birds, and possibly in some fishes, shows that it has arisen independently in different groups. The fact that in other insects and vertebrates the male is heterogametic suggests that female heterogamety must have been evolved from the XX♀ XY♂ type and not from forms with no unequal pair in either sex. Castle [50] is responsible for an interesting suggestion in this connection. He pointed out that the genetical results in female heterogamety could be explained on the assumption that the female condition is represented by X and the male by no-X. This assumption can be brought into line with the cytological facts, expressed in the ZW♀, ZZ♂ formula by the further assumption that Z=Y (*i.e.* inactive in determining sex) and W=X. However, no final solution of the problem is possible until more evidence is forthcoming.

The evidence reviewed in this chapter can leave no reasonable doubt concerning the validity of the sex-chromosome theory in all essential respects, although our knowledge of the subject is still very incomplete. It is apparent, further, that the sex-chromosomes only provide one of a complex of factors involved in sex determination. The other hereditary factors are borne by the autosomes and possibly by the cytoplasm, and therefore they are distributed equally to both sexes. The sex-chromosomes, on the other hand, are distributed unequally to the two sexes and consequently provide the mechanism which makes the whole complex either male producing or female producing. Only in this sense do they *determine* sex. These considerations lead to the conclusion that sex is the result of the interaction of a number of inherited factors or genes and of the environment ; being thus essentially similar to any other character. This genetical conception is not affected by cases of sex reversal occurring under special environmental conditions. These cases, on the contrary, furnish some of the most valuable evidence in favour of the sex-chromosome theory.

CHAPTER V

THE SEX-RATIO

General Application.—The sex-chromosome theory of sex-determination postulates the production of the sexes in approximately equal numbers since half the gametes of the heterogametic sex are male producing and half are female producing. The study of the sex-ratio therefore provides at once a test for the sex-chromosome theory and for disturbances of the chromosomal determination of sex which may arise naturally or experimentally. The sex-ratio, since it is capable of numerical expression and of mathematical treatment, yields most valuable data on these points. The statistical examination of the sex-ratio, though comparatively simple in so far as the mathematical processes involved are concerned, needs careful use, since incomplete analysis or faulty interpretation may entirely vitiate the results. The data employed in the present chapter will be chiefly mammalian on account of the greater volume available in comparison with those on other vertebrates, but the applications of the method, and many of the conclusions, are relevant to other forms. Unfortunately relatively little work has been done on the sex-ratio in vertebrates other than mammals, in which our knowledge is virtually confined to man and to the common domestic animals. Further work on birds and lower vertebrates may yield very important results and is much to be desired.

The sex-ratio may be expressed numerically in two or three different ways. The older workers usually expressed it as the number of males per 100 females. This form of expression permits of the accurate calculation of the probable error only for values near to 100. This disadvantage has

led recent workers to abandon the method in favour of either of the two following. The number of males may be expressed as a percentage of all cases or else the male ratio may be given as a decimal of unity. These two methods are equally good since they both convey the same information and permit of the accurate calculation of the probable error for all values provided the total number of cases is given. The probable error for the percentage of males is given by the formula

$$0{\cdot}6745\sqrt{mf/n}$$

where m and f are the percentages of males and females respectively, and n is the number of cases. The probable error of the difference between two male percentages is required in comparing two separate sex-ratios and is calculated from the formula

$$\sqrt{A^2+B^2}$$

where A and B are the probable errors of the two percentages to be compared. It is customary in comparing two sex-ratios to consider the difference significant when it is at least three times the probable error.

It is obvious that the 50 : 50 ratio expected on the assumption of the sex-chromosome theory may be disturbed in several ways. First it must be remembered that this expected ratio applies to the period of conception only and depends on the assumption that fertilisation by either an X- or a Y-bearing gamete is purely fortuitous. This expectation will not be fulfilled if there is any selection of either the male or the female determining gamete in preference to the other. Such selective fertilisation might be produced in several ways, all of which would result in a preponderance of one sex being produced at conception. First of all in the heterogametic sex there might be a tendency to produce more mature gametes with the X-chromosome than with the Y, or *vice versâ*. This tendency might be due to differential mortality of the gametes resulting from any cause, or, in forms in which the female is heterogametic, to a tendency for the X-chromosome to be

retained in the egg and the Y-chromosome extruded in the polar body, or *vice versâ*. Seiler [206] was able to influence by experimental means the extrusion of the Z-chromosome in the eggs of an insect (see p. 191). Another possibility arises in forms in which the male is heterogametic, since the sperms of one kind might be better adapted to reaching the egg than those of the other kind. For example, sperms with the X-chromosome might be supposed to possess an advantage and so be able to outstrip those without in the race to the ovum, or again, the presence of the X-chromosome might be an additional burden tending to hinder rather than to help. Finally, one kind of sperm might be better equipped to penetrate the egg than the other. It is difficult to estimate the importance of this factor since it is not practical to determine the chromosomal constitution of the fertilised egg, and consequently the actual ratio at conception cannot be observed directly. It is necessary to arrive at this ratio by observing the sex-ratio at some time after the sexual organs have differentiated and the sex can be identified anatomically. This may be done during embryonic life, which involves the destruction of the embryos, and, in mammals, of the mother also. It is therefore easier to determine the sex-ratio at birth or subsequently. Such ratios, however, require correction before they can be assumed to correspond with the ratio determined at conception by the sex-chromosomal constitution of the fertilised eggs. Two sources of error must be taken into account in this respect. First, the greater mortality of one sex than of the other occurring between conception and the time of observation of the sex-ratio. This unequal sex-incidence of mortality is known to be an important factor in determining the mammalian sex-ratio. Secondly, the reversal of sex as determined by the chromosomal constitution may take place and increase the number of one sex at the expense of the other. Such sex-reversal is unknown in the mammals and may be ignored as a possible factor influencing the mammalian sex-ratio, but it is of great importance in considering the ratios in many other vertebrates, especially fishes and amphibians. The further consideration of this disturbance of the sex-ratio

will be postponed to the chapters (XI and XII) specially devoted to the subject.

The two factors determining the mammalian sex-ratio at any given time are, therefore, (*a*) the initial ratio at conception, and (*b*) the amount and sex-incidence of mortality occurring between conception and the given time. Variation occurring in both these factors together may produce either a cumulative or a compensatory effect. This fact increases the difficulty of determining the relative importance of these two factors in reference to any given sex-ratio. Indeed such determination is often impossible.

It is convenient in dealing with the observed sex-ratios in the light of these theoretical conclusions to consider first the normal sex-ratio at birth, before birth, and after birth. Then the variations in the normal sex-ratio occurring as the result of various conditions will be more easily understood.

The Sex-ratio at Birth.—It is frequently necessary, owing to lack of data, to restrict the study of the mammalian sex-ratio to the three chief points in the life cycle : conception, birth, and maturity. The ratios at these three points are often called the primary, secondary, and tertiary ratios. The ratio at birth is the most suitable with which to begin because of the greater amount of data available. Reliable data concerning the ratio at maturity are only available for man, while the ratio at conception can be arrived at only, as already mentioned, by calculations based on the ratio at birth.

The most remarkable fact about the sex-ratio at birth is, as has been pointed out by Parkes [176] and others, its comparative constancy even in the most varied conditions. The ratio in man almost invariably approaches unity, but with a slight excess of males. Nichols [163] obtained a ratio of 105·5 males per hundred females, or 51·3 per cent. males from 447,019,579 births compiled for all parts of the world. The Registrar-General's Returns for England and Wales (1838–1914) show an average ratio of 104·0. Slight racial differences in the sex-ratio of human births appear to exist (for references, see [176]). The European and Asiatic races appear, on the whole, to show similar ratios, but Negro

races appear to have a lower percentage of males, while the Jews are credited with a higher male percentage. The reasons for these variations in the normal ratio are not clear. Parkes [176] gives the sex-ratios for domestic animals, recorded by various authors, in tabular form, but points out that these are not very reliable since they are founded on herd- and stud-book returns. The horse (96·5 to 99·7 males per 100 females) and the sheep (97·4 to 97·7) have the lowest ratios, while the dog (110·7 to 118·5) has the highest. The cow and the pig show wider variations, but appear to occupy an intermediate position.

The data for the mouse and the rat, though much less extensive, are more reliable since they are based on laboratory results. King [131] found a ratio per 100 females of 105·2±2·00 for the Albino rat and 85·8±2·68 for the Norway rat, while Parkes [172,175] found male percentages of 54·2±1·04 (1921–2) and 51·7±0·77 (1922–5) in his colony of Albino mice.

Pre-natal Mortality.—It is necessary, in order to obtain an idea of the sex-ratio at conception from that at birth, to estimate both the amount and the sex-incidence of pre-natal mortality. The amount of pre-natal mortality may be estimated by either of two methods, according to whether the dead embryo is removed by reabsorption or abortion. Abortion of one dead fœtus in polytocous animals would result, obviously, in the destruction of the whole litter. Consequently autolysis of the dead fœtus followed by more or less complete reabsorption is the rule in polytocous mammals and may occur frequently in early stages of monotocous forms. Abortion, on the other hand, appears to be the normal method of removal of a dead fœtus, especially when advanced in development, in monotocous forms, including man.

It is difficult to estimate the number of fœtuses which are reabsorbed, but rough results may be obtained by comparing the number of fœtuses *in utero* at autopsy or the number of young in the litter at birth with the number of corpora lutea in the ovaries. The two following tables summarise the results obtained by these methods by various authors. Table I shows the difference between the number of

fœtuses and corpora lutea and consequently does not cover the pre-natal mortality throughout the entire period of gestation.

TABLE I.—COMPARISON OF NUMBERS OF FŒTUSES AND CORPORA LUTEA (AFTER PARKES [176]).

Animal.	Normal fœtuses.	Atrophic fœtuses.	Corpora lutea.	Percentage mortality.
Pig . . .	267	49	396	32·5
,, . . .	3442	43	4480	23·3
Ferret . . .	1246	—	1643	24·2
Sheep . . .	101	8	116	12·9
Mouse . . .	76	—	82	7·3

Table II shows the average size of litter and the average number of corpora lutea and consequently covers the entire pre-natal mortality, since the still-births are included in most cases.

TABLE II.—COMPARISON OF AVERAGE SIZE OF LITTER AT BIRTH WITH AVERAGE NUMBER OF CORPORA LUTEA (AFTER PARKES [176])

Animal.	Size of litter.	Corpora lutea.	Percentage mortality.
Pig	12·0	20·0	40·0
Ferret	6·0	9·95	39·23
Rabbit	5·0	9·6	37·5
,, ...	6·9	9·11	18·33
Rat	6·7	10·0	33·0

Both these methods are inaccurate in so far as they do not exclude unfertilised ova, or identical twins arising from a single ovum. The occurrence of the former would tend to increase and of the latter to decrease the estimated pre-natal mortality. Therefore these factors would tend to be compensatory. These methods, since they involve the death of the animal, are not applicable to man. On the other hand, the data concerning the number of abortions compared to the number of normal births are almost all for man and furnish an index of the amount of pre-natal mortality. It is obvious that cases of very early abortion will remain unnoticed and unrecorded.

Using the data as they exist, various authors have attempted to estimate the amount of abortion and have mostly arrived at figures between 15 and 25 per cent. of all pregnancies. It is known that a large proportion, estimated at about 50 per cent., of the abortions take place in the first three months. The frequency of still-births, estimated at between 3 and 5 per cent. of all births, must be included in the data relating to the frequency of pre-natal mortality in man. Taking these figures, and the fact that very early abortions must remain unnoticed, into account it is obvious that the total amount of pre-natal mortality in man must be high.

The amount of pre-natal mortality in mammals generally is thus amply sufficient to cause profound modification of the sex-ratio at birth if the sex-incidence of the mortality does not approximate to equality. It is therefore a matter of much interest to determine the sex-incidence of pre-natal mortality. Unfortunately such determination is extremely difficult, especially in polytocous forms where reabsorption speedily renders identification of sex impossible. Some evidence may be obtained, however, from the sex-ratio of abortions and still-births. The data regarding the sex of human abortions appear to show a definite preponderance of males, and there is some evidence that this preponderance is greatest during the early months of pregnancy. More convincing data are available concerning the sex-ratio of still-births in man which appears to range between 124 and 144 males per hundred females. King [130] found a sex-ratio of 129·3 for still-born rats, which is remarkably similar to that for man. There are, therefore, good grounds for considering that pre-natal mortality falls most heavily on the male. The reason for this differential mortality is obscure. Many authors have attributed the higher mortality among male fœtuses to their greater size, requiring more nutrition and rendering birth more difficult. Since the mortality is highest during the early stages of gestation it is difficult to believe that the very slight size differences that could then exist play any important part. The hypothesis scarcely seems applicable even in the case of still-births, since the majority of the infants are dead

before labour starts. However, the real objection to this hypothesis is that the excess male mortality continues after birth, when slight size differences can have virtually no effect.

A more interesting explanation has been put forward by Huxley,[119] who suggests that recessive sex-linked deleterious factors are concerned. This is probable on *a priori* grounds, since the majority of recessives appear to be deleterious. Such factors, situated in the X-chromosome, would be less potent in the female, in which their normal allelomorph would be present, than in males where the X-chromosome is unpaired. This would account also for the different sex-ratios observed by different authors in various laboratory animals, for the mortality of males in inbred strains with few recessives would be less than in strains with many recessives. Unfortunately there is virtually no evidence either to confirm or refute this suggestion.

The Sex-ratio at Conception.—The occurrence of an appreciable amount of pre-natal mortality affecting the males more than the females indicates that the sex-ratio, which approaches equality at birth, must have shown a marked preponderance of males at conception. The proportion of males at conception must be higher than at any subsequent time. It is obviously impossible to determine directly the ratio at conception, but some attempt may be made to test the fœtal sex-ratio during the later stages of gestation. The most interesting data, provided by the work of Parkes [174] on the pig, are summarised in Table III.

Table III.—Sex-ratio of Fœtal Pigs according to Weight (after Parkes [176]).

Weight group.	No. of fœtuses.	Males.	Females.	Percentage males.
0–100 grms.	281	166	115	59·1±1·98
101–300 ,,	114	65	49	57·0±3·12
300+ ,,	188	100	88	53·2±2·45
Total . .	583	331	252	56·8±1·38

The ratio of 56·8±1·38 for all the fœtuses is distinctly higher than that of 49·56±0·065 for combined figures of pigs at birth. Moreover, the three size groups show an inverse correlation between the percentage of males and the stage of development, as indicated by the weight. Crew,[58] who also worked on the fœtal sex-ratio of pigs, confirmed Parkes' results. These figures indicate that the ratio at conception in the pig must approximate to 60 per cent. males, or 150 males per hundred females.

MacDowell and Lord [148] investigated the sex-ratio of litters of mice in which no pre-natal mortality had taken place, as shown by the correspondence between the number of young and of corpora lutea. They examined 523 young, comprising 68 litters, and found 261 males and 262 females. This equality ratio appears to show that the ratio at conception is not higher than at birth. It must be remembered, however, that the litters were selected as showing no pre-natal mortality and hence do not represent a random sample of conceptions. It has been pointed out that if males were less viable than females, litters with more females would tend to escape pre-natal mortality to a greater extent than those with more males, and would consequently form an undue proportion of the selected litters, thus weighting the results.

Taking the results as a whole it must be admitted that they are in favour of a substantial excess of males at conception in man and other mammals. This conclusion appears, at first sight, to be at variance with the sex-chromosome theory of sex-determination which postulates a ratio of equality at conception. It must be remembered, however, that this postulate depends on the further assumption that fertilisation by either an X- or a Y-bearing sperm is purely fortuitous. The sex-chromosome theory does not necessitate this assumption, but only implies that the X- and the Y-bearing sperms are produced in equal numbers. The high sex-ratio at conception in mammals may be explained, therefore, in accord with the sex-chromosome theory by assuming selective fertilisation favouring the X-bearing sperms. Unfortunately

there is insufficient evidence to show by what method this selective fertilisation is effected.

The Sex-ratio after Birth.—The post-natal changes in the sex-ratio throw little light on the theory of sex-determination, except in so far as they show that mortality continues to fall more heavily on the males than on the females. This is especially true at first, after which the sex-ratio of mortality falls until after puberty. The sex-ratio of mortality during the first year of life in England and Wales is shown in Table IV.

TABLE IV.—MORTALITY DURING FIRST YEAR OF LIFE BY AGE AND SEX. ENGLAND AND WALES (1913) (AFTER PARKES [176]).

Age.	Deaths per 1000.		Sex-ratio.
	Males.	Females.	
0–3 months	67·47	51·79	131·5
3–6 ,,	21·64	17·82	121
6–9 ,,	16·81	14·04	120
9–12 ,,	14·22	12·59	113
Total for year . . .	120·14	96·24	125

The only period of life when mortality falls heavier on the females than the males is between ten and twenty years of age, which is obviously due to the greater severity of puberty in the female. Both the sex-ratio and the percentage of mortality throughout life for England and Wales (1913) are shown in the accompanying Table V, together with the sex-ratio.

The excessive male incidence of mortality results in a decline in the ratio of males to females. The ratio, which at birth was 104, is reduced to 102 at the end of the first year. The decline continues steadily throughout life except for the years 15–20 and 45–55, when a rise occurs following the increased female mortality taking place during the previous five years at puberty and the climateric respectively. The number of females has become double that of males by the time extreme old age is attained.

Variations arising from Hybridisation and Inbreeding.—There is a good deal of evidence in favour of the view that

TABLE V.—POST-NATAL MORTALITY AND ESTIMATED POPULATION OF ENGLAND AND WALES, 1913, BY AGE AND SEX (AFTER PARKES [176]).

Age.	Mortality.		Sex-ratio (males per 100 females).
	Percentage.	Sex-ratio.	
0–5	3·24	113·4	101·0
5–10	0·30	100·7	99·9
10–15	0·18	93·3	94·2
15–20	0·27	104·9	98·5
20–25	0·32	115·3	89·7
25–30	0·28	117·5	81·4
30–35	0·47	119·6	
35–40	0·63	120·1	89·7
40–45	0·81	127·5	
45–50	1·10	132·5	92·2
50–55	1·51	132·0	
55–60	2·18	131·1	89·3
60–65	3·19	132·8	
65–70	4·55	128·0	79·4
70–75	7·62	121·0	
75–80	11·21	121·0	68·0
80–85	16·39	115·0	
85+	25·58	113·0	55·2

racial and specific crosses affect the sex-ratio of the offspring. Parkes [176] summarises the data relating to racial crosses in man by the generalisation that (*a*) crosses between white races produce an excess of males over pure white matings, (*b*) crosses between coloured races produce an excess of females over pure coloured matings, and (*c*) crosses between white and coloured races show an excess of females over pure matings of either race. King [126] showed that Albino mice crossed with Japanese waltzing mice gave a sex-ratio of 113·17 on 356 individuals, a ratio probably above that for pure matings of either species. The same author found that crosses of the wild Norway rat and Albino rat gave a ratio of 119·07 on 425 individuals, which is considerably higher than the normal ratio of 105·5 for the Albino rat. Sumner, McDaniel, and Huestis [213] found that sub-specific crosses of Peromyscus gave a sex-ratio of $104{\cdot}76 \pm 3{\cdot}41$, while pure matings gave $93{\cdot}27 \pm 2{\cdot}32$. These results show fairly definitely that hybridisation does affect the sex-ratio, but they throw no light at present on the manner in which this result is brought about.

Crosses between different species and genera often produce offspring of which those of one sex are normal, while those of the other sex are absent or rare, and, if present, are sterile and sometimes even intersexual. Many such inter-specific crosses are known in vertebrates and invertebrates, but the data for any given cross are small. Haldane [100] has made a critical analysis of the data and has formulated the rule that " When in the F1 offspring of two different animal races one sex is absent, rare, or sterile, that sex is the heterozygous sex." He believes that there is only one certain and a few doubtful exceptions to this rule.

The evidence concerning the effects of inbreeding in man on the sex-ratio is conflicting and inconclusive. Reliable and extensive data are provided, however, by King [129] on the rat. The sex-ratio of 3,256 young, produced by brother and sister matings for six consecutive generations without selection, was 108·6. This ratio is not convincingly different from the normal ratio of 105·0. It may be concluded, in consequence, that even this extreme degree of inbreeding produces no definite alteration in the sex-ratio. The ratio was modified in inbred strains in which the females were selected from litters showing either a preponderance of males or females, the former tending to produce an excess of males and the latter of females.

Seasonal Variations.—Considerable seasonal variations in the birth rate in man occur in many places. Several authors have claimed that these variations in the birth rate are correlated with slight variations in the sex-ratio, the proportion of males being higher at the seasons when the birth rate is low. More definite seasonal variations in the sex-ratio have been observed in rats and mice (see [176]). The reason for these variations is obscure, but it is probable that they are not due to variations in the pre-natal mortality, since, as Parkes has pointed out, this explanation would involve the assumption that the greatest pre-natal mortality took place during the optimum breeding season.

Variations arising from the Age of the Parent and the Number of Pregnancies.—The results, chiefly relating to man, concerning the influence of the age of the parents on the sex-ratio of the

offspring, are conflicting, but appear, on the whole, to point to a decline in the excess of males with increasing age of the mother. Parkes [171] has shown that pre-natal mortality increases with the age of the mother, and suggests that this is the cause of the correlated decline in the sex-ratio.

The number of the pregnancy, which is related to the age of the mother, also appears to affect the sex-ratio, as is shown for man in the accompanying table.

TABLE VI.—SEX-RATIO IN MAN ACCORDING TO NUMBER OF PREGNANCY (AFTER PARKES [176]).

Number of pregnancy.	Punnett.			Parkes.
	Burke's Peerage.	Torres Straits.	Murray Island.	Hospitals.
1	140·0	113·4	120·9	115·0
2	117·2	110·4	114·5	100·9
3 (+)	104·1	95·9	102·2	115·0
4 (+)	102·6	93·3	—	105·9
5 } 6 }	101·2	—	—	115·0
7 } 8 (+) }	102·7	—	—	97·2
9	100·0	—	—	—

These data show a decline in the proportion of males correlated with multiparity. Similar results have been observed with rats and mice. It is probable that this decline is caused by pre-natal mortality, which is known to increase with multiparity as well as with the age of the mother.

Combinations of Sexes in Multiple Births.—Multiple births may occur either occasionally in normally monotocous forms or normally in polytocous forms. The data concerning the former class of multiple births are almost entirely confined to man. The distribution of the sexes in the possible combinations of twins and triplets is interesting, since it does not correspond with the theoretical distribution calculated on the assumption that they are purely a matter of chance. The following table, based on Duncker's [69] work, shows the distribution in 1000 twins and 1000 triplets.

TABLE VII.—OBSERVED AND EXPECTED DISTRIBUTION OF SEXES IN HUMAN TWINS AND TRIPLETS (AFTER PARKES [176]).

Combination of sexes.	Observed distribution.	Expected distribution.	Difference.
Twins :			
2 ♂'s	326	261·7	+ 64·3
1 ♂–1 ♀	371	499·6	−128·6
2 ♀'s	303	238·7	+ 64·3
Total . . .	1000	1000·0	
Triplets :			
3 ♂'s	245	137·9	+107·1
2 ♂'s–1 ♀	285	387·1	−102·1
1 ♂–2 ♀'s	245	362·1	−117·1
3 ♀'s	225	112·9	+112·1
Total . . .	1000	1000·0	

This table shows that twins and triplets are much more frequently of one sex than they should be on a chance basis. This can be accounted for by polyembryony, since when two or more embryos arise from a single fertilised ovum and consequently have the same chromosomal constitution they will be of the same sex. The above data can, in fact, be used as an index of the amount of polyembryony in man, and suggest that about one-third of all twins and one-quarter of all triplets are monozygotic. The observed distribution of the sexes in man therefore supports the chromosomal theory of sex.

Bertillon [17] gives data which show that the number of uni-sexual, compared with bi-sexual, twins tends to decrease with the increasing age of the mother. Polyembryony is, in other words, more prevalent with young than with old mothers. The same author also finds slight racial variations in the amount of polyembryony.

The data concerning normally polytocous mammals are scarce. Sumner, McDaniel, and Huestis [213] found in Peromyscus that the observed distributions of the sexes in the possible combinations of the different sizes of litters corresponded very closely to the expected distributions on

a chance basis. Parkes [176] found that in the pig the litters with nearly equal numbers of males and females were more frequent than would be expected on a chance basis. Table VIII gives a summary of his data.

TABLE VIII.—COMBINATION OF SEXES IN PIG LITTERS (AFTER PARKES [176]).

Group.	Number of litters.		Difference.
	Observed.	Expected.	
Litters with females in excess. Extremes.	66	90·4	− 24·4
Litters with females in excess. Intermediates	520	551·2	− 31·2
Litters with sexes approximately equal	890	774·4	+115·6
Litters with males in excess. Intermediates	428	477·2	− 49·2
Litters with males in excess. Extremes.	57	67·4	− 10·4
Totals	1961	1960·6	—

The meaning of this tendency in the pig to produce equal numbers of the sexes in each litter is difficult to understand. It is clear, however, that polyembryony does not take place to an appreciable extent either in Peromyscus or the pig. Similar data for some polytocous forms, such as the opossum, in which polyembryony is known to occur would be of much interest.

Experimental Modification of the Sex-ratio.—Several attempts, based on the work of Pearl,[189] have been made to modify the sex-ratio in mice by administration of alcohol or narcotics to the male parent. Both Bluhm [19] and Danforth [62] obtained positive results which are summarised in Table IX.

TABLE IX.—EFFECT OF ALCOHOL, ETC., ON THE SEX-RATIO IN MICE.

Author.	Treatment.	Males.	Females.	Sex-ratio.
Danforth [62]	Controls	575	557	50·79±1·00
,,	Alcohol to male	210	164	56·15±1·73
Bluhm [19]	Controls	652	817	44·38±0·87
,,	Alcohol to male	182	149	54·98±1·84
,,	Yohimbin to male	185	154	54·57±1·82
,,	Caffeine to male	188	162	53·71±1·80

Parkes and Bellerby obtained similar results with alcohol administration, but MacDowell and MacDowell [149] obtained no effect on the sex-ratio. The evidence on the whole seems to warrant the conclusion that alcohol and narcotics administered to the male increase the percentage of males among the offspring. Since similar agents administered to the female do not affect the sex-ratio it may be concluded that the effect is produced by differential action on the X- and Y-bearing spermatozoa. In other words, the Y-bearing sperms tend to resist the action of the agents better than the X-bearing sperms, and more males are produced in consequence.

Parkes [173] obtained a remarkable effect on the sex-ratio of mice by submitting the male parents to doses of X-rays insufficient to produce sterility. The data are summarised in Table X.

TABLE X.—EFFECT ON THE SEX-RATIO PRODUCED BY X-RAYS ON THE MALE PARENT (PARKES [173]).

Time of conception in days after irradiation of male.	Number of offspring.		Sex-ratio.
	Males.	Females.	
0–4	79	54	59·4±2·87
5–18	48	95	33·6±2·66
19+	118	99	54·4±2·27

The normal ratio for animals bred under precisely similar conditions was 51·6±1·24. The experimental ratio for the first four days after irradiation is therefore probably significantly different. The increase in the male percentage which it exhibits may be due to a greater mortality of the X-bearing sperms, as compared with the Y-bearing sperms, which were mature at the time of irradiation. Such a differential mortality might be expected from the slightly greater size of the X-bearing sperms, rendering them a better target for the X-rays. The subsequent swing back of the ratio to a heavy excess of females, for conceptions between five and eighteen days after irradiation, is undoubtedly significant and is even more difficult to explain. Parkes suggests that this may be due to the X-rays causing

non-disjunction of the X- and Y-chromosomes at the reduction division. This would result in the formation of XY-bearing sperms, which would produce females, as well as the normal X-sperms. The final ratio of 54·4 is not significantly different from the normal and need not be taken into account. However these results are to be interpreted they show that the mammalian sex-ratio is capable of experimental modification. It is possible that further work will result in the discovery of a more effective method of eliminating one type of spermatozoon and provide a useful and practical means of controlling the sex of offspring.

It may be concluded that the study of the mammalian sex-ratio, probably the oldest method of attacking the problem of sex-determination, thus provides weighty evidence in favour of the sex-chromosome theory.

CHAPTER VI

THE ORIGIN OF THE PRIMORDIAL GERM-CELLS AND THE FORMATION OF THE GERMINAL RIDGES

THE formation of the gonads begins at a very early stage in the embryonic development of all vertebrates. Synchronously, or even before the appearance of the rudiments of the gonads, germ-cells are differentiated. The origin of these germ-cells and the problem of whether or not the definitive ova and spermatozoa are derived entirely, or at all, from them has been and is still very controversial. The problem is of first-rate biological importance and has a distinct bearing on the subject of this book. In consequence, a large part of this chapter will be devoted to it, and the remainder will deal with the early differentiation and organisation of the gonads up till the time when they differentiate into either ovaries or testes.

The Origin of the Primordial Germ-cells.—The earlier workers considered that the germ-cells in vertebrates originated exclusively from the cells of the peritoneal or germinal epithelium covering the gonads. This was the view held by Waldeyer [223]. Nussbaum,[164] as early as 1880, expressed the opinion that the primordial germ-cells arise at a very early stage of development before the differentiation of the germinal ridges. Further work by many authors soon showed that the primordial germ-cells, probably in all vertebrates, arise in the wall of the yolk-sac and migrate into the forming germinal ridges. This view of the extra-regional origin and early migration of the primordial germ-cells met with opposition but gained increased acceptance with the appearance of Weismann's theory of " the continuity of the germ-plasm." This theory demanded the early separa-

tion of the germ-cells from the soma and emphatically denied the somatic origin of germ-cells during later life. Consequently belief in the early origin of the primordial germ-cells and the derivation of all the definitive germ-cells from them became part of the creed of Weismannian teaching, and, as such, remained unquestioned for some time. Evidence has been increasing steadily which appears to show that germ-cells do arise from other sources than the primordial germ-cells. This view, however, does not cast doubt on the early extra-regional origin and migration of the primordial germ-cells, which may be considered well established although denied by some embryologists, but it raises the question of whether the definitive germ-cells originate from them or from other sources. These problems can be dealt with best by considering first the embryological evidence relating chiefly to the origin, migration, and ultimate fate of the primordial germ-cells, and then the evidence, partly experimental, dealing with the origin of the definitive germ-cells. The origin and migration of the primordial germ-cells have been described in all vertebrate classes.

Cyclostomes.—The primordial germ-cells in the Cyclostomes have been described by Wheeler [228]. They appear in young embryos at the stage when the lateral plates of mesoderm are separating from the primitive endoderm. They are numerous, large and heavily loaded with yolk which gives them a characteristic appearance resembling small blastomeres. Consequently they are easily distinguishable from the surrounding cells. These primordial germ-cells originate in the endoderm of the middle of the trunk and migrate into the mesoderm on each side throughout the greater part of the posterior region of the trunk. The primordial germ-cells are amœboid, to which fact they owe their power of migration. They retain their distinctive cytological characters and do not exhibit mitosis during migration. Subsequently the primordial germ-cells reach the neighbourhood of the base of the mesentery, immediately beneath the aorta, by way of the mesoderm. The yolk spheres then disappear and the primordial germ-cells enter on a phase of active mitosis. The

subsequent changes in the developing gonads require further elucidation.

Pisces.—The primordial germ-cells originate very early in fish. They occur in very young embryos of Selacians in the endoderm on each side where the embryonic and extra-embryonic regions merge (Woods [250], Beard [11]). They are scattered in the endoderm of the lateral and ventral walls of the enteron in Ganoides (Allen [5]). They first appear in small numbers in the primary endoderm before the differentiation of the mesoderm and endoderm, in Teleosts, but increase in number in later stages (Dodds [66]). They resemble the primordial germ-cells of Cyclostomes, but have large, clear nuclei, and exhibit specific and individual variation in number. They migrate from the endoderm through the splanchnic mesoderm, or between it and the endoderm, to the base of the mesentery, beneath the aorta. Then they move out into the germinal ridges, which form one on each side between the mesentery and the mesonephros. Although the majority appear to reach the germinal ridges many are lost *en route* and may be seen in the myotomes and even in the neural canal, where doubtless they degenerate. Once within the germinal ridges the primordial germ-cells absorb their yolk content and undergo mitoses. They are, at this and subsequent stages, surrounded by epithelial cells, which, should they transform into germ-cells, would be indistinguishable from the germ-cells of extra-regional origin. Consequently it is virtually impossible to arrive at a definite conclusion, on morphological grounds, as to the ultimate fate of the primordial germ-cells and whether or not secondary germ-cells arise from the peritoneal epithelium covering the germinal ridges. Failure to realise the inadequacy of the morphological method for providing a solution to these problems has led to much useless controversy.

Amphibia.—Considerable diversity of opinion appears to exist in the literature concerning the mesodermal or endodermal origin of the primordial germ-cells. The recent work of Bounoure [23] appears to show definitely that the primordial germ-cells in both Urodeles and Anurans originate in the endoderm. The primordial germ-cells are large cells possess-

ing the typical characters ; a large, clear nucleus and cytoplasm loaded with yolk-spheres. Pigment is also present in the cytoplasm in some forms. The primordial germ-cells in the Urodeles soon separate from the endoderm and enter the mesoderm, forming a strand on each side immediately beneath the Wolffian duct. Subsequently, with the formation of the mesentery, these strands unite into a median cord. The primordial germ-cells in the Anurans arise in the endoderm on each side of the enteron and migrate from there to the median dorsal line of the endoderm, where they form a single cord or crest, which becomes separated by the mesoderm forming the base of the mesentery. The median unpaired ridge of primordial germ-cells in both Urodeles and Anurans soon separates into the two lateral germinal ridges, covered by peritoneal epithelium and projecting into the body cavity between the Wolffian duct and the mesentery, on each side.

Reptiles.—The origin and migration of the primordial germ-cells is extraordinarily clear in reptiles. They arise in the yolk-sac endoderm on each side where it joins the extra-embryonic blastoderm in the turtle, Chrysemys (Dustin [70]). They are formed very early and can be traced back to stages in which the yolk-sac endoderm is barely differentiated (Allen [4]). They are large cells with large, clear nuclei and cytoplasm laden with yolk-spheres. They migrate through the endoderm or splanchnic mesoderm to the base of the mesentery and thence into the germinal ridges on each side. During migration they retain their characteristic structure and never divide, but the yolk-spheres are absorbed soon after they enter the germinal ridges. Many of the primordial germ-cells degenerate without reaching the germinal ridges, others become lost and may be seen in the splanchnic mesoderm or endoderm after the others have attained their definitive position. These stray cells persist for some time and finally disappear, but their exact fate is not known.

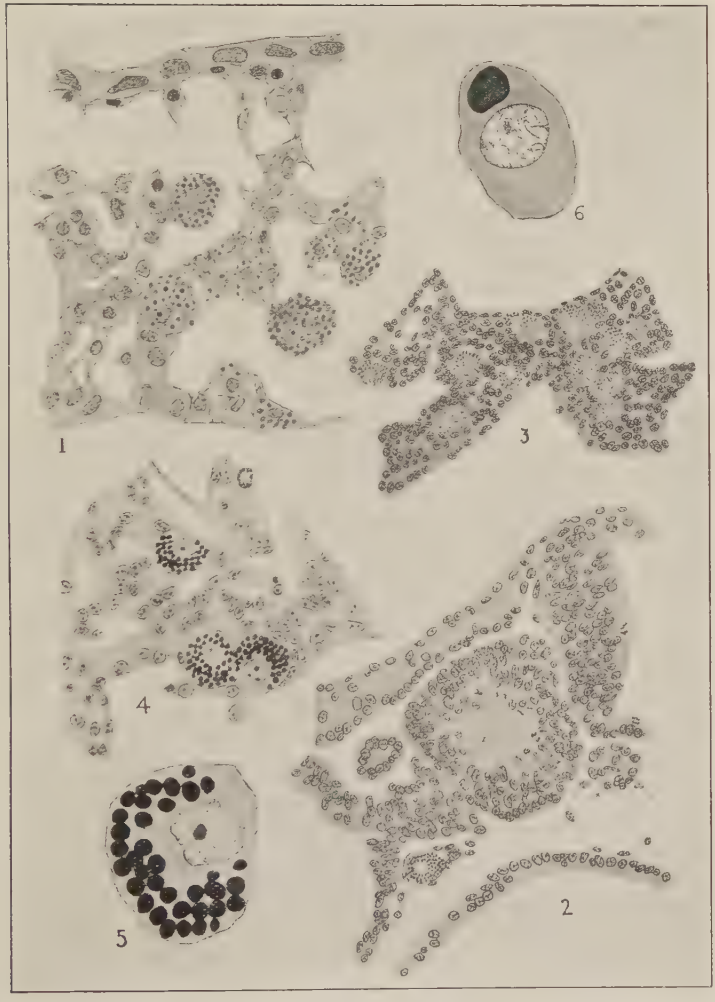
The conditions in the reptile, Sphenodon, are still clearer and our knowledge is more complete [218]. The primordial germ-cells (Pl. VIII) in this form arise in a crescentic area in front of the head of the embryo at an early stage.

PLATE VIII

Primordial Germ-cells of Sphenodon

1. Three primordial germs-cells, full of small yolk-spheres, in the yolk-sac endoderm of the area in front of the head of an embryo in the primitive streak stage. ×240. 2. A primordial germ-cell is seen in the branch of the vitelline vein shown below the nephrotome. × 100. 3. Primordial germ-cells in the mesoderm above the groove of the midgut. × 100. 4. Two primordial germ-cells in the germinal ridge and one still in the mesoderm above it. × 240. 5. A single primordial germ-cell from the same stage as Fig. 4. × 530. 6. A single primordial germ-cell from a later stage. Most of the yolk has been absorbed, but some still remains in the form of a single large globule. The nucleus has entered on the prophase of the heterotypic division and is in the lepto-zygotene stage. × 530. (After [218].)

PLATE VIII



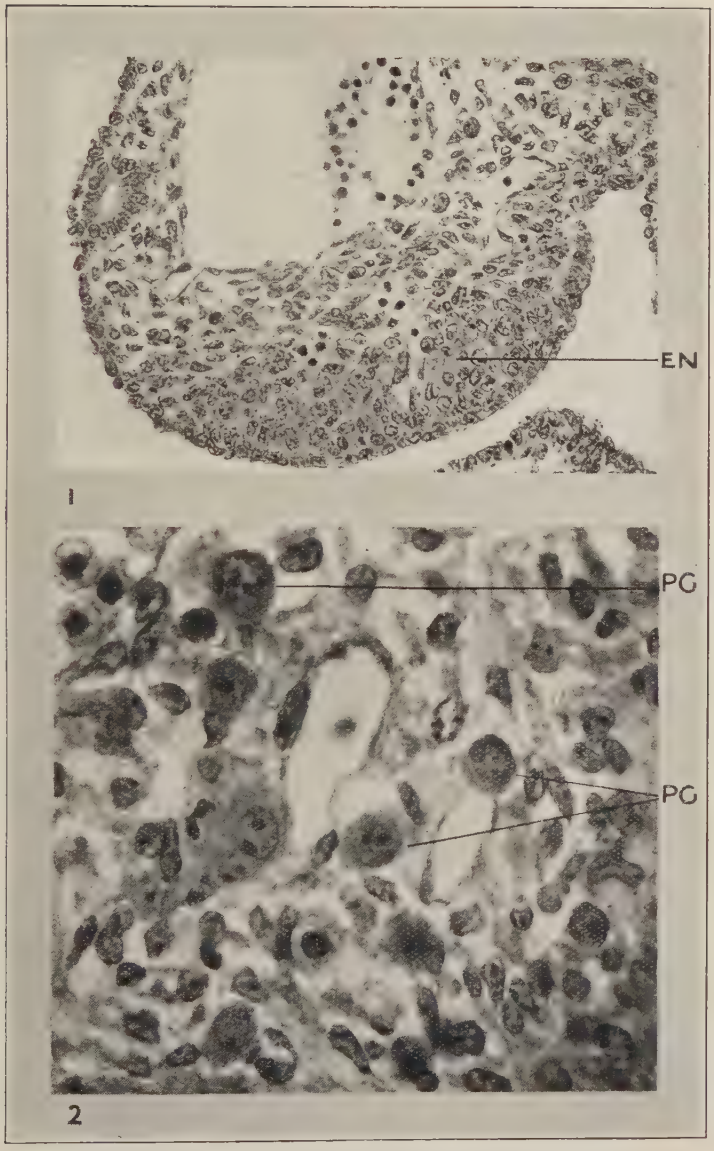
The endoderm cells as they are formed are laden with yolk in the form of one or more large spheres. These yolk-spheres break down, both in the cells which are definitive endoderm and in those which are primordial germ-cells. The yolk-spheres break up into smaller spheres of very variable size, which gradually fragment still further and are finally completely absorbed in the endoderm cells. They break down into a cloud of small spheres which are approximately equal in size and which are not further absorbed in the primordial germ-cell. At first the forming primordial germ-cells differ from the endoderm cells only in this respect, both being of approximately the same size and irregularly stellate in shape. Soon the primordial germ-cells become ovate or spherical, and are still further distinguished from the endoderm cells by the progressive decrease in the yolk content of the latter. The nuclei of the primordial germ-cells do not undergo striking modification but remain comparatively small and dense. The formation of the primordial germ-cells continues for some time, and the outer borders of the crescentic area in which they form somewhat extend. As soon as the overlying mesoderm develops many of the primordial germ-cells are found in it, frequently in the blood islands. Active migration does not begin, however, until shortly before the time when the embryonic heart begins to beat. At this and the succeeding stages many primordial germ-cells are to be found in the vitelline veins and arteries, a few in the dorsal aorta, and even in the heart. Others occur in the extra-embryonic splanchnic mesoderm and endoderm. The germ-cells migrate through the mesoderm or are carried by the blood-stream, when circulation is established, until they reach the region of the mesonephros, when they pass through the intervening mesenchyme and enter the germinal ridges, which only begin to form at this stage on each side between the base of the mesentery and the mesonephros. It can be seen that the primordial germ-cells long antedate the germinal ridges, which are not recognisable as such until after some of the primordial germ-cells have entered them. Many of the primordial germ-cells appear to get lost during migration. Such stray cells are

PLATE IX

Development of the Gonads of the Mouse

1. Germinal ridge of a twelve-day embryo, showing the formation of the epithelial nucleus (EN) by the medullary proliferation. ×245. 2. Six primordial germ-cells (PG) in the body wall above the germinal ridge of a ten-day embryo. × 735. (After [28].)

PLATE IX



to be found occasionally in many parts of the body, even lying in contact with the neural tube. They are specially common in the mesoderm of the head, even in front of the eye, where they appear to get jammed in the smaller vessels owing to their large size. Their presence in the venous system suggests that they migrate up the vitelline veins with the blood-stream, but, probably, also through the tissues of the splanchnopleur by their amœboid activity. During migration and until after their entry into the germinal ridges the primordial germ-cells retain their characteristic structure and heavy load of yolk-spheres. After their entry into the germinal ridges they undergo a phase of active mitoses during which the yolk-spheres are absorbed. Subsequently the cells enter on the prophase of the reduction division, and in some cases the characteristic yolk-spheres can still be observed in germ-cells in synizesis. This observation proves that these cells are definitely germ-cells, although it still leaves in doubt the question of whether they ever give rise to definitive ova or spermatozoa.

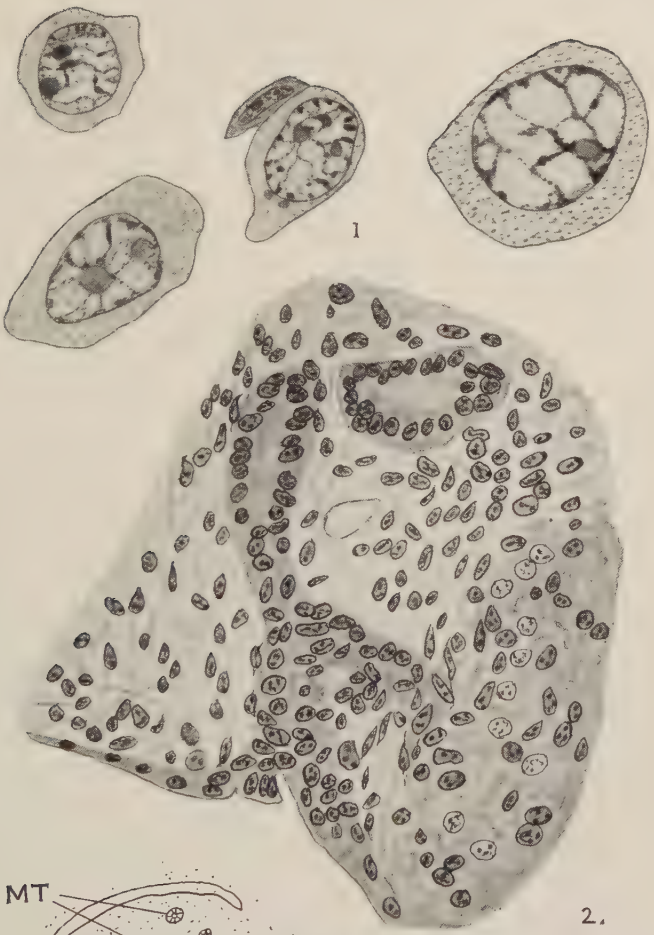
Birds.—The origin and migration of the primordial germ-cells in birds are remarkably similar to those of Sphenodon. They arise in the chick (Swift [214]), at a very early stage, in the yolk-sac endoderm in a crescentic area, anterior and antero-lateral to the embryo at the margin of the area pellucida, and occupy the space between the endoderm and ectoderm. Later they wander into the mesoderm and enter the blood vessels at the stage when they are forming. They are carried in the blood to all parts of the embryo, but collect in the vessels of the splanchnic mesoderm in 20-22 somite embryos. Subsequently they disappear from the blood-stream and are found in the splanchnic mesoderm near the angle of the cœlom and the dorsal aorta. They wander from thence through the mesoderm into the germinal ridges. Many are lost on the way to the germinal ridges and apparently degenerate. The primordial germ-cells are large, averaging about 16 μ in diameter, with large nuclei and yolk-spheres in the cytoplasm. The centrosome, with two centrioles, is close to the nucleus and is surrounded by the archoplasm and by the Golgi bodies, in the

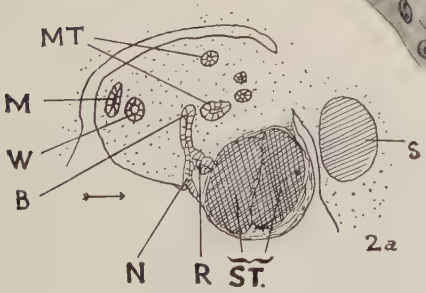
PLATE X

Development of the Gonads of the Mouse

1. Primordial germ-cells. An epithelial cell is shown attached to the centre figure for comparison. × 1240. 2. Drawing of a nephrostomial canal in the region of the gonad of a twelve-day male embryo. The rudimentary capsule of Bowman (B) can be seen. Between it and the peritoneal epithelium a narrow down-growth (R) connects the nephrostomial canal (N) with the spermatic tubules (ST) in the testis. This is the primordium of a rete tubule. 2*a* provides a key to the adjacent parts. M, Müllerian duct; MT, mesonephric tubules; S, suprarenal; W, Wolffian duct. × 600. (After [28, 29].)

PLATE X





form of granules or short rods (Woodger [249]). The rod-shaped mitochondria are scattered through the cytoplasm. The yolk-spheres gradually disappear from the primordial germ-cells but remain until long after they have disappeared from the other embryonic tissues.

The site of origin and migration by way of the blood-stream are remarkably similar in the fowl and in Sphenodon, an interesting point in view of the primitive character of the latter and the reptilian ancestry of birds.

Mammals.—The exact site of origin of the primordial germ-cells in mammals is by no means so clear as in other vertebrates. There is, however, evidence that they form in the walls of the yolk-sac, presumably in the endoderm, and that their origin is thus in line with other vertebrates. About the time when the germinal ridges are forming, extra-regional primordial germ-cells occur in the yolk-sac stalk, the mesentery, and the neighbourhood of the forming germinal ridges (Jenkinson [122], also [28]); a distribution which is consistent with the interpretation that they are migrating from the yolk-sac to the germinal ridges, although they might also be interpreted as formed in the germinal ridges and wandering out from them. The latter interpretation is not admissible, however, in the case of other vertebrates. The primordial germ-cells of mammals are similar in structure and appearance to those of birds and reptiles, but contain relatively little yolk (Pl. IX, Fig. 2, and Pl. X, Fig. 1). All the primordial germ-cells have either reached the germinal ridges or degenerated on the way by the time the latter are well formed. Subsequently extra-regional germ-cells cannot be found in any part of the embryo.

The results described show conclusively that primordial germ-cells occur in all vertebrate groups, originating from the endoderm and subsequently migrating into the germinal ridges when they begin to form. This conclusion must be admitted by any competent person who is familiar with the literature. It is probable that these cells enter on the prophase of the reduction division, but the morphological method has proved insufficient to elucidate their ultimate fate. Some

evidence is forthcoming, however, in one or two special cases.

Many authors have attempted to show that oocytes are formed in the adult ovaries of vertebrates, but the results have not been convincing owing to the failure to find the early stages of the prophase of the reduction division. Some authors have even gone so far as to state that oocytes are formed from epithelial cells without passing through these characteristic nuclear phases, but this view has gained no measure of general acceptance. The Lemurs provide, however, much more convincing evidence. Gerard [91] has described nests of young oocytes, with nuclei in the typical phases of the meiotic prophase, in the adult ovaries of Galago. Prof. J. P. Hill and Dr. A. Subba Rau have drawn the author's attention to a similar condition of the adult ovaries of Loris and have courteously allowed him to examine their material. There can be no doubt that these oocytes are actually undergoing the prophase changes in the adult ovaries, but further research is necessary to determine whether they originate by the transformation of epithelial cells or from oogonia, which may have arisen from primordial germ-cells and have persisted in the ovaries without entering on the prophase changes of the meiotic division until adult life. The latter supposition is rendered more probable by the observation of de Winiwarter [234] that the formation of oocytes in the immediate vicinity of the hilum is delayed and may still be in progress after it has ceased elsewhere. Such oocytes, still exhibiting meiotic prophase stages, were observed in the ovaries of young cats shortly after puberty.

Other evidence is provided by cases of sex-reversal in adult female frogs and birds, to be described in Chapters XI–XII. At least in some cases the spermatogonia and spermatocytes formed in these sex-reversed ovaries appear to arise from epithelial elements and not from pre-existing germ-cells.

Experimental Evidence concerning the Origin of the Definitive Germ-cells.—The problem of whether the primordial germ-cells give rise exclusively or in part to the definitive germ-cells demands experimental solution. Such a solution has not yet been achieved although recently several attempts

have been made to attack the problem experimentally and some results of interest have been obtained.

Some years ago Reagan [198] tried a crucial experiment on the fowl by attempting to remove the primordial germ-cells before they reached the rudiment of the gonad. He excised, at a very early stage of incubation of the egg, the area of extra-embryonic blastoderm in front of the head in which all the primordial germ-cells are believed to be formed. Unfortunately very few of the large number of operated embryos survived, and none more than five days. At this stage the gonads were found to lack germ-cells. This work was never repeated and is open to the criticism that secondary germ-cells might reasonably be expected to be formed only considerably later than any of the embryos survived.

Recently Humphrey [115] has carried out a similar experiment on Amblystoma. In early stages of this form the primordial germ-cells are all situated in the lateral mesoderm, immediately below the Wolffian duct, between somites 7 and 16. He removed them from one side by excising this strip of mesoderm together with the Wolffian duct and the overlying ectoderm. More than half the embryos survived after this treatment and were kept alive for long periods. In some cases stray germ-cells were missed which later gave origin to gonad nodules on the operated side. In many cases they appear to have been completely removed, for no germinal ridge was subsequently formed on that side, and consequently germ-cells were not reformed later. In other cases, not yet fully described, Humphrey states that a sterile germinal ridge was formed on the operated side, in which germ-cells developed later. This experiment, if it is substantiated, is of importance in that it aims at the removal of all the primordial germ-cells before they have reached the gonad rudiment and not at the extirpation of the gonad as a whole.

Conflicting results have been obtained in mice by two separate methods. It has been shown that female mice, whether immature or adult, exposed to a standard dose of X-rays became completely sterile in the vast majority of cases after about forty days [36, 37, 38]. Histological examination of a

series of ovaries from these mice revealed the fact that all the small oocytes degenerated and disappeared within a few hours after irradiation. A number of the larger oocytes degenerated more slowly, but a few of them appeared to mature in a normal manner, and were ovulated. When all had done so or had degenerated, which took about forty days, the ovaries became completely sterile. These sterile ovaries, although devoid of follicles, had an apparently normal germinal epithelium and a cortex composed chiefly of remnants of corpora lutea and epithelial elements. Anovular follicles were formed from the germinal epithelium of the sterilised ovaries of young animals. Moreover, they retained the power of producing certain essential ovarian hormones (*i.e.* œstrin) and of regulating the œstrous cycle. It was also shown that these females, if mated after irradiation, could, and often did, become pregnant and produce and rear normal litters during the first forty days. Some of these animals that had themselves become pregnant after irradiation were killed upwards of forty days after X-ray treatment and the ovaries were found to be completely devoid of follicles and oocytes. Other animals were kept for as much as five months after irradiation, a very long period in the life of the mouse, but they remained completely sterile and in none was any reformation of germ-cells observed. This experiment admits of only two explanations. Either the X-rays effected some permanent, but quite invisible, change in the germinal epithelium which robbed it of all power of subsequently producing germ-cells, although some of those present at the time of irradiation were able to complete development, or else germ-cells are not produced in the mouse ovary after puberty. The latter explanation certainly appears the more probable.

Another line of evidence is provided by experiments on double ovariotomy in mice (Davenport [65], Parkes, Fielding and Brambell [188]). Both ovaries were completely removed, together with the Fallopian tube, and the adjacent portion of the fat body from a large number of mice. In about 90 per cent. of these animals ovarian tissue, containing healthy oocytes, was regenerated. The ovarian tissue was regenerated in the

old ovarian region, but, beyond the fact that it could not have been formed from the ovaries previously removed, which were preserved and were shown, by complete serial sections, to have been taken out intact and *in toto*, its origin could not be identified. This result appears to show conclusively that germ-cells can be formed in the adult mouse after complete removal of the gonads, and is diametrically opposed in this respect to the results obtained with X-rays.

The contention that oocytes are not formed in the adult ovary is supported by the work of Lipschütz and Voss [140-142] on partial ovariotomy in cats and rabbits. It was found that when one ovary and the greater part of the other were removed the remaining fragment underwent compensatory hypertrophy. This fragment, moreover, produced a normal number of mature follicles ovulating at each œstrus. It was found that after a number of œstrous periods the number of small oocytes in the ovarian fragment was greatly reduced, obviously owing to the rapid depletion caused by ovulation. It was shown that this depletion of oocytes was not due to operative or post-operative effects, since similar fragments in animals in which one ovary was left intact did not exhibit it. This observation, which needs confirmation and careful statistical analysis, appears to show that oocytes are not produced in the adult ovary.

In conclusion, it must be admitted that the experimental results obtained up to the present time are conflicting and puzzling as regards the main problem, although themselves very interesting. They open up, at least, a new approach to this difficult field and may stimulate further work.

The Formation of the Germinal Ridges.—The formation of the germinal ridges or anlaga of the definitive gonads begins simultaneously with the arrival of the primordial germ-cells in and beneath the peritoneal epithelium covering their future site. The development of the germinal ridges begins anteriorly and extends in a posterior direction. The anterior extremities are consequently always in a more advanced stage of development than the posterior, the difference being marked in many forms. The germinal ridges are situated, one on each

side, between the base of the mesentery and the Wolffian duct, on the ventral surface of the developing mesonephros. They project into the body cavity and extend throughout the lumbar region of the trunk. They are continuous and do not exhibit any metameric arrangement in the majority of vertebrates. Traces of metamerism are apparent, however, in some amphibians, but nothing approaching the complete segmental arrangement in Amphioxus is found in higher forms.

The first stage in the formation of the germinal ridges consists in a thickening of the peritoneal epithelium over their site. The primordial germ-cells, having completed their migration, arrange themselves in and beneath these bands of thickened or germinal epithelium. The germinal epithelium then begins to proliferate cells from its inner surface. This medullary proliferation may be in the form of distinct short medullary cords, as in the cat, rabbit, etc. (de Winiwarter [232-236]), or may be more or less continuous over the surface, as in the mouse [28]. The primordial germ-cells become included among the epithelial cells, forming this medullary proliferation. The proliferation continues for some time until the germinal ridges are well defined and project markedly into the body cavity. Then proliferation gradually stops and elements from the underlying mesenchyme grow down into the epithelial mass. Some of these mesenchymatous cells form connective tissue trabeculæ between the blocks of epithelial cells, accentuating their arrangement in separate cords. Others grow down on each side between the germinal epithelium and the medullary mass which it has proliferated, and form a thin continuous layer of connective tissue, the primitive tunica albuginea. The germinal ridges at this time (Pl. IX, Fig. 1) therefore consist of a medulla composed of epithelial cords containing the dividing germ-cells, and separated by thin connective tissue trabeculæ, into which capillaries penetrate. The medulla is surrounded by the primitive tunica albuginea which separates it from the overlying germinal epithelium, now quiescent. The whole germinal ridge by this time has become partly constricted off, by the deepening of a lateral longitudinal groove on each side, from the mesonephros to which it is attached by a broad

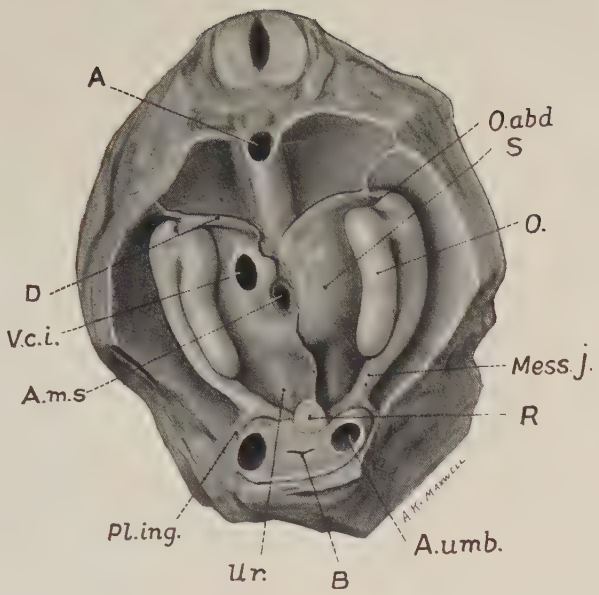
mesentery. The gonad, having attained this stage of development, is ready to differentiate into either an ovary or a testis under the influence of the sex-determining stimuli. The subsequent development is essentially different in the two sexes and will be dealt with separately in the next chapter.

PLATE XI

Dissections of 12½-day p.c. Mouse Embryos

1. Female embryo. The long, narrow ovaries are seen attached to the small mesonephric fold. Swellings in the dorsal body wall indicate the position of the developing kidneys and suprarenal bodies. The cephalic extremity of each mesonephric fold, showing the ostium abdominale tubæ, is attached to the diaphragm, which is cut across, by the short plica diaphragmatica. × 19·5. 2. Male embryo. The testes are much thicker in proportion than the ovaries of the female embryo from the same uterus (Fig. 1). The loops of the spermatic tubules can be seen as slight ridges on the surface of the testis on the right-hand side of the picture. The ureters are seen as two ridges on the dorsal body wall, and the inguinal folds are clearly visible. × 18·7.

Guide letters :—A, aorta ; A.m.s., superior mesenteric artery ; A.umb., umbilical artery ; B, bladder ; D, diaphragm ; GT, genital tubercle ; Mess.J., mesonephric fold (tubal portion) ; O, ovary ; O.abd., ostium abdominale tubæ ; Pl.d., plica diaphragmatica ; Pl.ing., plica inguinalis ; R, rectum ; S, ridge formed by the developing suprarenal and kidney ; T, testis ; Ur., ureter ; V.c.i., inferior vena cava ; V.h.a., hemiazygos vein. (From [29].)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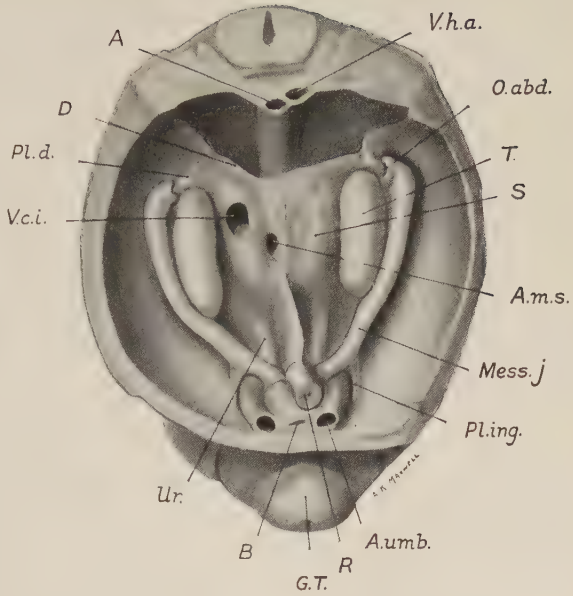
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PLATE XI

CHAPTER VII

THE DIFFERENTIATION OF SEX AND THE DEVELOPMENT OF THE GONADS

THE differentiation of the indifferent gonads into ovaries or testes and their later development in the Amniotes have been the subject of much research since the work of de Winiwarter and Sainmont [236] on the development of the ovaries in the cat appeared in 1909. Unfortunately most of the work on Anamniotes antedates this and is therefore out of date and difficult to correlate with our knowledge of higher forms. Moreover, among the Amniotes the development of the gonads in mammals is known more accurately and in greater detail than that of reptiles, and even than that of birds. It seems advisable, therefore, to make the development of the gonads in mammals the basis of this chapter and to supplement it, where necessary, with a description of the conditions in other groups.

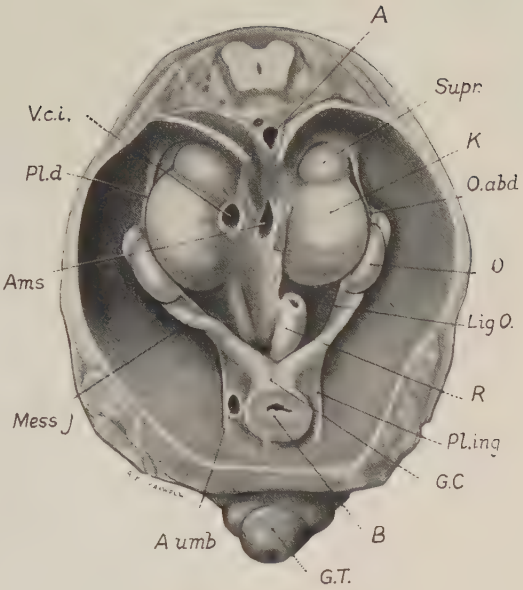
The Differentiation of Sex.—Among mammals and birds the indifferent gonads of male embryos begin to differentiate into testes before those of females differentiate into ovaries. Consequently at this stage the ovaries can only be distinguished from the testes of the same age by comparison, since they are still in the indifferent stage. The differentiation of the testes in the mouse, and probably in all mammals, is accompanied by a more rapid growth in diameter of the testes as compared with the ovaries (Pls. XI and XII). It is possible, in fact, to distinguish the testes from the ovaries by their greater diameter as soon as they can be identified histologically. It is necessary, however, to use embryos from the same litter for comparison, so as to ensure their age being

PLATE XII

Dissections of Fifteen-day p.c. Mouse Embryos

1. Female embryo. The ovaries are being pushed laterally by the developing kidneys, which are now distinguishable from the suprarenal bodies. The plica diaphragmatica is long. The ostium abdominale tubæ is clearly visible. The ligamentum ovarii and plica inguinalis are clearly shown. The junction of the tubal portions of the two mesonephric folds to form the genital cord is visible just in front of the bladder. In this specimen only one umbilical artery could be distinguished. × 12·3. 2. Male embryo. The testes are much larger and more ovoid than the ovaries of the female embryo from the same uterus (Fig. 1). The loops of the spermatic cords can be seen as ridges on the surfaces of the testes. The testis on the right of the picture is approximately in its original position, but that on the left is undergoing a torsion about the mesonephric fold and is also being displaced laterally by the developing kidney. × 13·4. (After [29].)

Guide letters :—GC, genital cord ; K, kidney ; Lig.O., ligamentum ovarii ; Lig.T., ligamentum testis ; Supr., suprarenal. Other letters as in Plate XI.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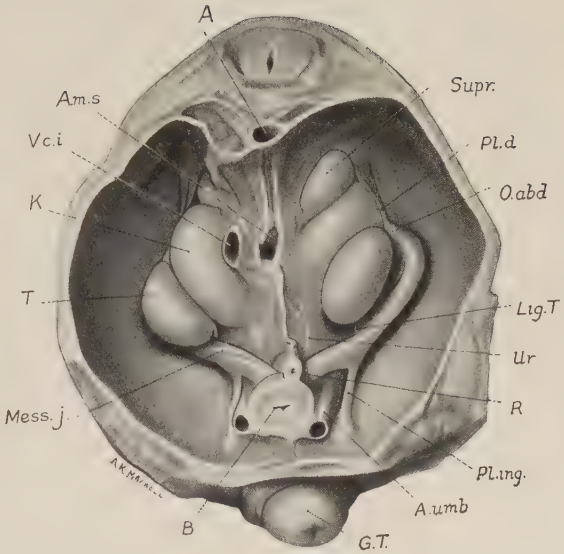
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PLATE XII

the same, since the ovaries begin to differentiate very soon after the testes. The differentiation of the testis before the ovary is probably true of all vertebrates, but our knowledge of lower forms is insufficient to arrive at a definite conclusion. This observation obviously does not apply to frogs in which the male gonads differentiate into ovaries first and then transform into testes.

The Development of the Testes.—The Formation of the Spermatic Cords.—The medullary cords of the indifferent gonad give rise directly to the spermatic cords of the testis. The downgrowth of mesenchyme cells from the mesonephros form, as already mentioned, trabeculæ which divide the blocks or cords of epithelial cells. At the same time the cords become definitely arranged in loops with their free ends pointing towards the hilum of the gonad. These loops are few in number and simply arranged in the mouse (Fig. 8, Pl. XIII, Figs. 2 and 3). The mesenchyme cells provide a coat of connective tissue around each, thus forming the walls of the spermatic cords. Within each spermatic cord the germ-cells increase rapidly by mitosis. The spermatic cords grow and become intertwined during the further growth of the testes. Gradually the undifferentiated epithelial cells and the germ-cells, many of which have by this time entered upon the prophase of the heterotypic division, become arranged around the wall of the cord in a layer several cells thick. This layer constitutes the spermatic or seminal epithelium and consists of germ-cells and epithelial cells, the latter of which are attached directly to the wall of the spermatic cord. These epithelial cells constitute the nurse or Sertoli cells to which the maturing sperms become attached and from which they probably absorb nutriment. The Sertoli cells are thus homologous to the epithelial cells of the ovarian follicles. The germ-cells consist of spermatogonia and spermatocytes, but do not give rise to the spermatids or ripe sperms until puberty. The primary spermatogonia are attached to the wall of the spermatic cord between the Sertoli cells, while the secondary spermatogonia and the later stages in spermatogenesis are nearer the centre of the cord. The spermatic cords develop

PLATE XIII

Development of the Gonads of the Mouse

1. Ovary of a 13½-day embryo showing many of the germ-cells in synapsis. × 138. 2. Testis of a twelve-day embryo. The germinal epithelium is cut off by the development of the tunica albuginea (TA) from the forming spermatic tubules. It has thickened (GE) along the ventral border of the gonad, owing to the occurrence of an incipient cortical proliferation. × 136. 3. Testis of a 13½-day embryo. The spermatic tubules are well formed and the germinal epithelium is so thin that it can scarcely be distinguished on the outside of the thick tunica albuginea. The Wolffian duct is larger and the Mullerian duct smaller than in the female embryo of the same age (Fig. 1). × 122. (Figs. 1 and 2 after [28].) Guide letters as in Plate X.

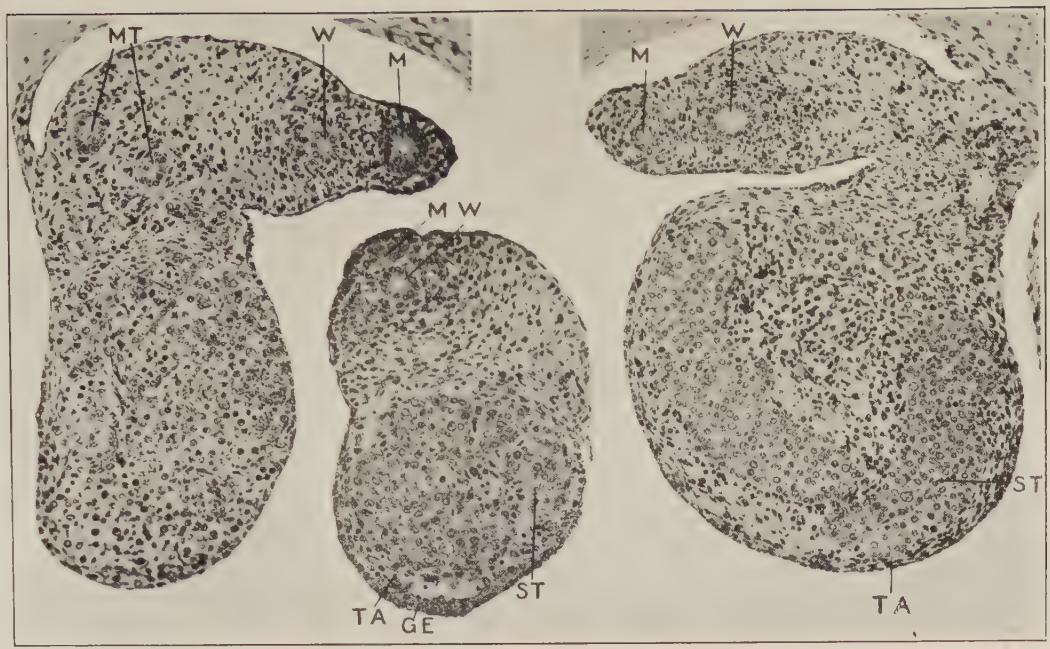

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PLATE XIII

a lumen about the time when the efferent ducts are formed, and are known subsequently as the spermatic tubules. The diameter of the spermatic tubules varies greatly with the degree of activity and consequent thickness of the seminal epithelium. Moreover, the bulk of the entire testis largely depends on the degree of activity in the spermatic tubules. This is shown by

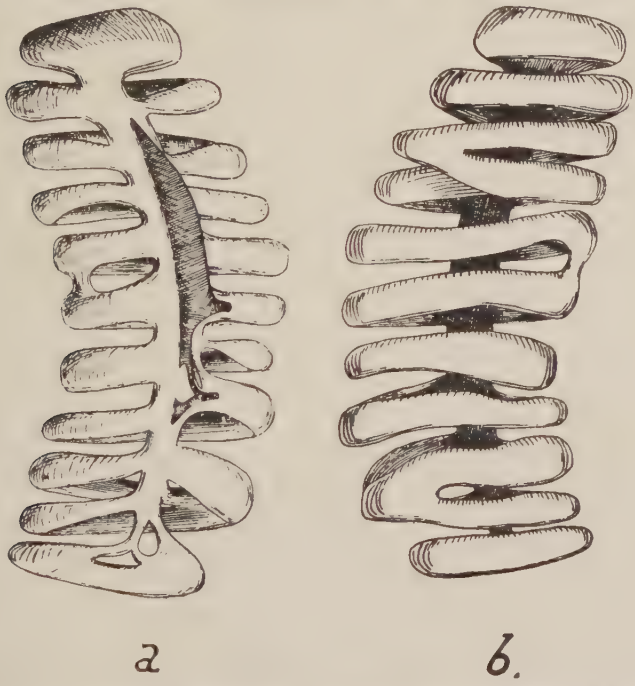


FIG. 8.—Model of the seminal tubules in the left testis of a mouse embryo. Dorsal (*a*) and ventral (*b*) views. × 130. (From de Burlet and de Ruiter [45].)

the fact that the huge increase in size of the testes of birds and other animals during the breeding season is almost entirely due to the hypertrophy of the spermatic tubules which accompanies the onset of active spermatogenesis.

The walls of the fully formed spermatic tubules are thin and are composed of several layers of fibrous connective tissue which is richly supplied with capillaries.

The spermatic acini or cysts in Amphioxus and the Cyclostomes, which have no efferent ducts, do not unite into definite tubules but remain as closed pockets surrounded by thin walls of connective tissue.

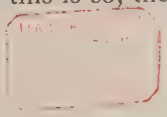
Interstitial Tissue.—The interstices between the spermatic tubules are occupied by loose connective tissue, the stroma, in which the larger blood vessels of the testis run. Interstitial cells are also present in small islands between the tubules. These cells are large and glandular in character and are said to be responsible for the production of the male hormones. The amount of interstitial tissue varies somewhat in different physiological conditions and with the age and functional activity of the testis. Their origin is disputed, some authors maintaining that they arise from modified stromal cells while other authors consider they are epithelial cells of the medullary proliferation which were not included in the spermatic cords. Unfortunately they have often been described as homologous to the so-called "interstitial" cells of the ovary, but the evidence for this assumption is very inadequate.

The Formation of the Tunica Albuginea.—The thin primitive tunica albuginea of the indifferent gonad persists without interruption and thickens to form the tunica albuginea of the adult testis. The tunica albuginea forms a dense, strong coat of very fibrous connective tissue around the whole testis. It is relatively thin in most vertebrates, but is much thicker in mammals in which the scrotal position of the testes renders them more liable to damage and more in need of effective protection. The tunica albuginea varies in thickness in different mammals and is very strongly developed in some.

The Germinal Epithelium.—The germinal epithelium is completely isolated from the medullary cords by the development of the primitive tunica albuginea. Proliferation consequently stops and the germinal epithelium becomes thin and cubical. It becomes active again in the ovaries and proliferates the cortical cords, the ingrowth of which destroys the primitive tunica albuginea. The germinal epithelium in the testes does not become active again after the formation of the

primitive tunica albuginea, but becomes thin and remains sterile and inactive throughout life. It is so thin in the adult testis of mammals that it is difficult to discern on the outside of the tunica albuginea the sparse flattened epithelial cells composing it. The mouse, however, exhibits an interesting phenomenon [28]. After the formation of the primitive tunica albuginea, when the gonad has definitely begun to differentiate into a testis, the germinal epithelium thickens along the ventral border of the gonad and proliferates a little (Pl. XIII, Fig. 2). This thickened band of epithelium does not, however, penetrate the primitive tunica albuginea but remains outside it. This thickening is identical in appearance with the beginning of the cortical proliferation of the ovary and occurs at the same stage. It is obvious that it is an abortive cortical proliferation occurring in the testis and is homologous to that which forms the cortex of the ovary. This abortive proliferation persists for a short time, then stops and gradually disappears, probably owing to being stretched out thin by the growth of the testis. This transient structure is of much interest in connection with hermaphrodite gonads of mammals and will be referred to in this connection in Chapter X.

Onset of Spermatogenesis.—Some time before puberty the seminal epithelium in the spermatic tubules enters on a period of active spermatogenesis. This phase of activity is not destined to produce mature spermatozoa but is overcome by a wave of degeneration which destroys large numbers of germ-cells. It is claimed that in some forms the germ-cells are completely destroyed and disappear from the testes for a short space of time. This does not appear to be the case in most forms, in which some germ-cells are always visible in the spermatic tubules. Nevertheless, even in these all the germ-cells may be destroyed, although not simultaneously. The opponents of the view that the primordial germ-cells produce definitive spermatozoa maintain that they are all destroyed at this time and that the germ-cells found in succeeding stages are secondary and have arisen from the epithelial cells in the tubules. Whether or not this is so, the wave of degeneration



is followed by renewed activity of the seminal epithelium resulting in definite spermatogenesis.

Spermatogenesis continues from puberty throughout life, but gradually slows and finally stops with age. It continues in men long after the age at which the menopause occurs in women.

The spermatids when they begin to elongate attach themselves by the side on which the acrosome develops to the Sertoli cells lining the wall of the spermatic tubules. The maturing spermatozoa are consequently arranged in dense tufts, each attached to a Sertoli cell, in the seminal tubules. Their heads lie side by side with the tips of the acrosomes embedded in the Sertoli cells and the tails projecting freely into the lumen of the tubule. When mature the spermatozoa free themselves from the Sertoli cells and congregate in the lumen of the tubule from whence they escape through the efferent ducts.

The Urogenital Connection and the Origin of the Rete.— An elaborate system of efferent ducts by which the spermatozoa are liberated is developed in Selachians, Amphibians, and Amniotes. It is formed by the development of a network of fine canals, the rete testis, which unite the spermatic tubules with a definite number of the mesonephric tubules in the anterior end of the mesonephros. The anterior region of the mesonephros, concerned in this urogenital connection, constitutes the epididymis, while the posterior region, which is purely excretory, forms the definitive kidney in Selachians and Amphibians, but degenerates during embryonic life and is only represented by the rudimentary and functionless paradidymis in adult Amniotes.

The origin of the rete canals been has the subject of much research and controversy. They appear to arise in Selachians (Brachet [26]) by the union of the nephrostomial canals of the anterior mesonephric tubules directly with the extremities of the spermatic tubules in the hilum of the testis. This union is effected after the degeneration of the nephrostomes which are present at first. Among the Amphibians the rete appears to be formed by the condensation of elements in the

region of the hilum which unite with Malpighian corpuscles and grow down from them into the testis at a very early stage before the spermatic tubules are formed. The spermatic tubules then form in connection with them (Witschi [239, 247]).

The origin of the rete in Amniotes is known in much greater detail, but much diversity of opinion exists. Some authors consider that it is formed by the condensation of mesenchyme cells in the mesonephros which unite with the Malpighian corpuscles and then with the spermatic tubules (Firket [81]). Others consider that it is formed from epithelial cells of cœlomic origin which constitute a rete blastema in the hilum of the gonad (Felix [79], Wilson [231], de Burlet and de Ruiter [45]). The most general view, however, appears to be that it is formed by downgrowths from the Malpighian corpuscles, the nephrostomial canals, or the nephrostomes themselves. A large number of workers, including de Winiwarter and Sainmont on the cat [236], de Winiwarter on the rabbit [232] and man [233], and Bovy on the mouse [25], consider that outgrowths from the walls of the Malpighian corpuscles unite with the spermatic cords and give origin to the rete canals.

Allen traces the rete to "peritoneal funnels" (nephrostomes) in the pig and rabbit [2], and in Chrysemys [3]. He states that the rete is formed by the union of "funnel-cords" (nephrostomial canals) with evaginations from the capsules of Bowman. The funnel-cords are derived from the "peritoneal funnels" (nephrostomes) of the Malpighian corpuscles. Fraser [83] describes the origin of the rete in Marsupials from a core of cells lying within the genital ridge anterior to the rudiment of the gonad. This core of cells, at least in some forms, is derived from the nephrostomial canals which have lost their connection both with the cœlomic epithelium and with the Malpighian corpuscles, and have united into a continuous mass. It was found [29] in the mouse that the rete originated from cords growing down from the nephrostomial canals (Pl. X, Fig. 2), between the nephrostomes and the Malpighian corpuscles, and Bovy [25], while denying the presence of nephrostomes, also attributed the origin

of the rete to downgrowths, but considered that these formed from the Malpighian corpuscles themselves. Taking the evidence as a whole it appears to favour the view that the rete originates from downgrowths from either the walls of the Malpighian corpuscles or else from the nephrostomes or their canals.

Brachet [26] has suggested that the open nephrostomes in primitive ancestral forms provided the channels by which the spermatozoa, shed into the cœlom, escaped to the exterior. This interesting and attractive hypothesis appears more probable when it is remembered that in Amphioxus and the Cyclostomes the spermatozoa are actually shed into the cœlom and that this primitive condition is retained in the females of even the Amniotes. If this were so it would be easy to understand how the rete arose originally from the nephrostomes of the mesonephros.

The spermatic tubules, where they are united with the rete, are often fused together in small groups into the straight tubules or tubuli recti in which spermatozoa are not formed and which lead directly into the rete canals (Pl. XIV, Fig. 1). The region of the hilum in the testis, where the rete unites with the straight tubules, is known as the mediastinum. After the urogenital connection is established the Malpighian corpuscles on the mesonephric tubules concerned degenerate and disappear while the tubules themselves persist and convey the spermatozoa from the rete into the Wolffian duct or vas deferens. The mesentery or ligament atttaching the testis and epididymis to the dorsal body wall is known as the mesorchium.

The Development of the Testis in Amphibia.—The development of the testis in Anurans is similar in outline to that in mammals but differs in several minor respects. Brief description of these is necessary for a proper understanding of the changes involved in sex-reversal, which will be described in Chapter XI.

The development of the testis in the frog has been described by Witschi [239] in the Alpine race of *Rana temporaria*. The gonad in the 23 mm. larva, just before sex differentiates, is in the form of a hollow ridge. The germinal epithelium is only

slightly thickened and encloses the germ-cells. This thin epithelial layer encloses the central primitive gonad cavity. Five to seven cords or strands of cells grow down from the mesonephric blastema at the base of the genital ridge into this cavity, which they fill. These strands are arranged serially from the anterior to the posterior end, and may indicate an incipient segmental arrangement of the gonads. Even at this early stage the future testes are distinguishable from the ovaries by the more rapid growth and greater thickness of the strands. The first stage in the differentiation of the testis is marked by the migration of the germ-cells, and of those epithelial cells which are destined to form the follicle cells, across the primitive gonad cavity. The germinal epithelium, having ceased proliferating, remains as a simple thin peritoneal investment of the developing gonad. The germ-cells and follicle-cells rearrange themselves as nests embedded in the mesonephric strands (Pl. XX, Fig. 3). These nests transform into the spermatic tubules by the development of a lumen, at first in the form of an irregular cleft, in each. The mesonephric strands give rise to the rete and vasa efferentia and so establish the urogenital connection. The testes grow very slowly in this race of frogs until the fourth season. Then the spermatogonia increase by active mitosis and the spermatic tubules consequently become convoluted. The maturation stages follow immediately and spermatozoa are formed. This development differs from that in the mammals, chiefly in the early development of the mesonephric strands which will form the rete. Further research on the origin of these strands appears desirable. Another striking difference is the presence of the large gonad cavity which compels the germ-cells and accompanying epithelial elements to migrate across it from the periphery to the centre. It is obvious that these germ-cells and follicle-cells are homologous to the medullary cords of the mammalian testis.

Certain other races of frogs normally or under certain abnormal conditions do not exhibit this direct form of testis development, but go through a more or less transitory female stage. These examples properly belong to the class of sex-

reversals and will be described in the chapter devoted to the subject.

The development of the gonads in the toad as described by Ponse [194] exhibits a more definite segmental arrangement. The indifferent genital ridge breaks up into three segments; an anterior or progonad, an intermediate or mesogonad, and a posterior or metagonad. These three primordia develop separately both spatially and chronologically. The progonad, which develops very precociously, differentiates into an incipient atypic ovary which constitutes the larval Bidder's organ. It soon degenerates almost completely. The mesogonad starts to develop at the time of metamorphosis. It differentiates also into an incipient ovary, which is destined to form the greater part of the definitive organ of Bidder. After metamorphosis, at the time when the metagonad starts to differentiate, the mesogonad undergoes a certain amount of degeneration. The metagonad alone differentiates into the definitive gonad and forms the testis in the male and the ovary in the female. This development in the male shows in a remarkably clear manner that a time factor is involved in the appearance of the male-determining stimulus. The segmental arrangement in both sexes is interesting and serves to confirm the conclusion that the primitive vertebrate had segmental gonads.

The Development of the Ovary.—The gonads of female embryos remain in the indifferent condition for a short time after the testes have differentiated. During this time they can only be distinguished negatively by comparison with testes of embryos of exactly the same stage of development (Pl. XI). Soon the ovaries begin to show signs of renewed activity of the germinal epithelium, which begins to thicken and to proliferate again. This second proliferation (Pl. XIII, Fig. 1) gives rise to the cortical cords which constitute the first positive signs of the differentiation into an ovary.

The Medullary Cords.—The growth of the cortical cords results ultimately in the degeneration of the medullary. The retrogression of the medullary cords does not set in immediately after the differentiation of the ovary, but is preceded by a

phase of development and differentiation, the duration of which depends on the species. During this development the oocytes in the medullary cords pass through the prophase changes of the heterotypic division, and may become surrounded by single-layered follicles. They enter on the growth stage in many cases and may attain a considerable size. Sooner or later fat-droplets appear in the oocytes and they degenerate completely. The medullary cords are then formed of epithelial elements only and are completely sterile. They atrophy still further and are only represented by a few strands of cells in the medullary region of the adult ovary. It is possible that some of the elements of the medullary cords become included in the cortex and give origin to " interstitial " cells. The islet cells of the bird's ovary have been described as originating in this way (Fell [77]).

The Primitive Tunica Albuginea.—The primitive tunica albuginea of the indifferent gonad, which gives rise to the definitive tunica albuginea of the testis, is perforated by the ingrowth of the cortical cords and is gradually broken up and entirely destroyed. The mesenchyme cells which compose it are carried into the ovary around the cortical cords and, together with others growing down from the hilum, constitute the connective tissue trabeculæ which partially separate the cortical cords and subsequently form the chief elements in the stroma of the adult ovary.

The Formation of the Cortex.—The cortical proliferation gives rise to thick, usually discrete, cords of epithelial cells, enclosing many germ-cells. The cords as they grow into the ovary break up the primitive tunica albuginea as described and gradually compress the medullary cords. They form in this way a compact cortex which constitutes the greater part of the ovary. Their growth continues for a considerable time, until after birth in all mammals and even until the approach of puberty in some. The germ-cells enter on the prophase of the heterotypic division soon after they are included in the growing cortical cords (Pl. XIII, Fig. 1). Consequently the oocytes in the deeper parts of the cortex, which were formed first, are in more advanced stages of development than those in

the more peripheral regions. A simple follicle of epithelial cells is formed around each oocyte by the time the nucleus has entered on the resting or dictyate stage. Some of the oocytes enter on the growth stage soon after birth, but the majority or all of these are destined to degenerate like those of the medullary cords, before puberty. This wave of growth and subsequent degeneration is a striking phenomenon in most birds and mammals and takes place some time before puberty. It has been suggested that it results in the degeneration of all the surviving primordial germ-cells and that only germ-cells of secondary origin are left after it is over. This precocious wave of growth in birds and mammals and probably other forms, which is always abortive, must be of some phylogenetic significance. One is tempted to surmise that it represents a precocious formation of functional ova in some primitive ancestral vertebrate. The subsequent degeneration of these oocytes in living forms is comparable to that of the spermatocytes in the immature testis, and both may be due to the evolution of a postponed sexual maturity in the individual.

The distinction between the medullary and the cortical cords, which is very clear in the cat, rabbit, man (de Winiwarter[232, 233, 236]) and many other forms, is not invariably well defined. It is not seen in the ovary of the mouse, but its existence can be inferred by comparison with the testis. It should be remembered, however, that this and many other peculiarities of embryo mice result in all probability from their rapid development and small size and are to be considered as secondary modifications to meet these special conditions and not as primitive characters.

The germinal epithelium is said to become active again after the proliferation of cortical cords has stopped and to produce a third and final set of cords in some mammals (*e.g.* cat, rabbit, and man (de Winiwarter)). These cords are in the form of invaginations of the epithelium (Fig. 9) which invade the cortex and are said to be the exclusive source of the definitive germ-cells. Their formation begins some time after birth and continues until puberty. During this time the oocytes in the cortical cords are said to degenerate completely

like those of the medullary cords. Substantiation of the regular occurrence of these epithelial invaginations and further researches to determine whether they occur in other mammals is desirable.

The Definitive Tunica Albuginea.—The definitive tunica albuginea of the ovary is formed from mesenchyme cells which have grown down from the hilum in a similar manner to those which formed the primitive tunica. Its formation takes place after the cortical proliferation has stopped. The subsequent

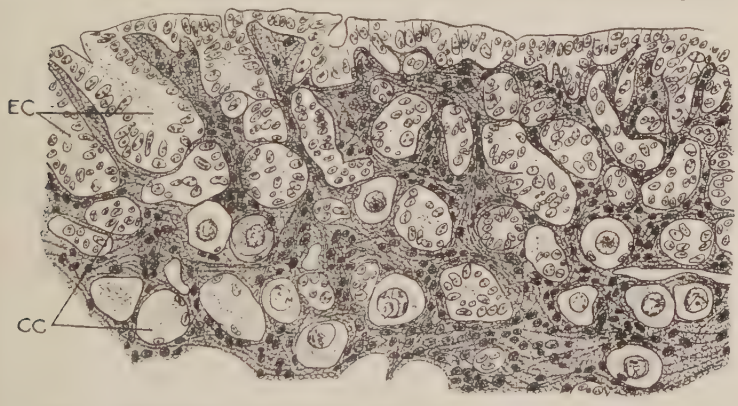


FIG. 9.—Epithelial invaginations (EC) and cortical cords (CC) in the ovary of a rabbit six weeks old. × 240. (Modified from de Winiwarter[232].)

ingrowth of the epithelial invaginations disturbs and perforates it but does not destroy it, and soon it becomes completely reorganised.

The tunica albuginea of the adult ovary is composed of fibrous connective tissue cells, forming a coat around the ovary immediately beneath the germinal epithelium. It varies greatly in thickness, being very thin in some forms, such as the Amphibia, and thick in others (*e.g.* most mammals). It is, however, never so well developed as that of the testis. The tunica albuginea gradually thins over the maturing follicles and finally ruptures at these points to allow of the escape of the ovum at ovulation.

The Epithelial Proliferations in other Vertebrates.—The third proliferation, giving rise to the epithelial invaginations,

appears to occur, as has been stated, only in mammals. Both cortical and medullary proliferations, however, occur in the development of the ovaries of birds and reptiles. The cortical proliferation in these vertebrate groups gives rise then to the entire cortex and contains the definitive germ-cells. It is probable that the formation of the ovarian cortex in Amphibians also may be divided into medullary and cortical proliferations, although Witschi, in his later papers on *Rana sylvatica*, does not make the distinction.

This author has described [239, 247] the differentiation of the ovary in frogs. The indifferent gonad which is destined to form an ovary can be distinguished frequently by the slower growth of the mesonephric strands or rete cords which are hollow at their distal extremities. The germinal epithelium continues to thicken after this stage, unlike that in the male, chiefly owing to the increase by mitosis of the germ-cells. These become arranged in nests by the time the larvæ have attained a length of 30-35 mm. The nests are formed first in the distal or ventral portion of the gonad. The oogonia in the nests undergo a few simultaneous mitotic divisions and then enter immediately upon the reduction stages. Many of the first-formed nests degenerate about the time of metamorphosis. After the prophase stages are completed the oocytes become surrounded by their follicles and enter upon the second growth period. Yolk, however, is not formed until the third or fourth year. Meanwhile the spaces which were appearing in the mesonephric strands at the time of differentiation, increase in size and unite to form a sac in each strand ; the secondary cavities of the ovary (Pl. XX, Fig. 1). These constitute the 5 to 7 endothelial sacs which occupy the centre of the adult ovary and fill the entire cavity enclosed by the cortex.

The development of the testis already described, furnishes a key to the interpretation of that of the ovary. Apart from it the homology of the ovarian sacs with the rete ovarii of higher forms would not be obvious. It is probable that the thickening of the germinal epithelium of the ovary before sexual differentiation is comparable to the medullary proliferation

and the subsequent thickening to the cortical proliferation of the mammalian ovary. The degeneration of the earlier-formed oocytes at the time of metamorphosis is reminiscent of the remarkable waves of degeneration observed in the immature ovaries of birds and mammals.

The distinction between cortical and medullary proliferations has not been made clear in lower forms. It is possible that they are distinct in Selachians and other fish and that, since there is little or no recent work on the subject, the earlier workers failed to differentiate between them. It may be, on the other hand, that only a single proliferation, probably representing the medullary proliferation of higher forms, occurs in their ovaries as it almost certainly does in Cyclostomes and probably in Teleosts. It seems probable, therefore, that in primitive forms only the medullary proliferation occurred and that the cortical and the third proliferation were gradually and successively evolved as additions to them. It is difficult to imagine why these supplementary epithelial proliferations in the ovary should be necessary or desirable in higher forms. Perhaps they were evolved in connection with a later attainment of sexual maturity in the life cycle. This is purely speculative, however, and more extended researches on the comparative embryology of the gonads in the various groups of vertebrates are necessary before any conclusions can be drawn.

The Stroma and so-called "Interstitial" Cells.—The mesenchymal cells which grow down into the ovary from the hilum during its development give rise to trabeculæ of connective tissue which partly separate and support the ingrowing epithelial cords. This connective tissue framework becomes considerably augmented during development and forms the stroma of the adult ovary. The stroma occupies most of the space between the follicles in the cortex of the ovary. It is entirely of mesodermal origin and is composed of connective tissue rich in fibroblasts. It becomes increasingly fibrous during later life and composes the major part of the ovary in old individuals after the menopause. The character of the stroma and its amount, relative to that of the follicles, varies

greatly in different species. Blood vessels, lymph channels, and nerves supplying the ovary traverse the stroma.

The stroma contains the so-called interstitial cells of the ovary. The term " interstitial " has been used very loosely and is commonly applied to any glandular elements which occur in the stroma irrespective of their origin. These cells vary considerably in structure and appearance. They are characterised as a rule by their large size, as compared with the stroma cells, round or polygonal shape, cytoplasm more or less loaded with fat or lipoid vacuoles, and large round nuclei. Their origin has been much disputed and has been assigned by various authors to cells of the epithelial cords which have not participated in the formation of follicles, to cells of the follicular epithelium of follicles that have degenerated, and in mammals to the cells of corpora lutea, formed either in atretic or ruptured follicles, which have retrogressed. Some authors maintain that they arise from the theca interna cells of degenerated follicles or retrogressed corpora lutea. The last view suggests that they originate from connective tissue cells, since it is probable that the theca interna cells are of this derivation, while all the other sources mentioned are ultimately traceable to epithelial cells. The most probable explanation is that there are several distinct types of cells described as " interstitial " and that they originate in different ways. It is desirable, therefore, that the term " interstitial cell " as applied to the ovary should be used tentatively and should be qualified, when used, by a description of the characters and, wherever possible, of the orgin of the particular cells in question. Some authors have gone so far as to consider the interstitial cells of the ovary homologous to the much more definite testicular interstitial cells, but for this assumption there is no justification.

The large, clear *islet cells* which occur singly or in small groups in the thecæ of the follicles and stroma of the ovaries of birds have been included in the term " interstitial," and have been called also " luteal cells." They have been shown to originate in the embryo from the epithelial cells of the retrogressing medullary cords [77]. Fell [78] is of opinion that

they are formed also in older ovaries from degenerating follicles.

The interstitial cells in mammals vary greatly in different forms. They are very plentiful, large and vacuolated in the ovary of the rabbit where they are said to originate from old corpora lutea. The normal mouse ovary is devoid of such cells, but the stroma contains many epithelial cells similar to those in the germinal epithelium. These cells are not normally large or vacuolated, but should be included in the term "interstitial." They originate from cells of the medullary and cortical proliferations which have not been included in the follicles. It has been shown, however, that these cells can transform first into luteal cells, similar in structure and function with those of the corpus luteum, and then into highly vacuolated cells closely resembling the interstitial cells of the rabbit ovary. This remarkable transformation in the ovary of epithelial cells which are not incorporated in follicles can be effected by X-rays [37] or by the injection of suitable extracts of the anterior pituitary [179]. These results indicate that epithelial cells in the ovary retain the power of transforming into these different types of cells under the influence of suitable stimuli irrespective of their location. It suggests that interstitial cells may originate in a normal ovary from any of the several different kinds of epithelial cells present, such as membrana granulosa, lutein, etc., without exhibiting any difference in their ultimate structure. Such a solution may well be true and would go far to account for the diversity of opinion as to the origin of interstitial cells in the ovary.

An endocrine function has been attributed often to the interstitial tissue of the ovary. It is not improbable that they are responsible for the production of the hormone *œstrin* referred to in the next chapter, and they may also be concerned in the production of other hormones, the existence of which is suspected. It is probable, therefore, that they play a very important part in ovarian physiology.

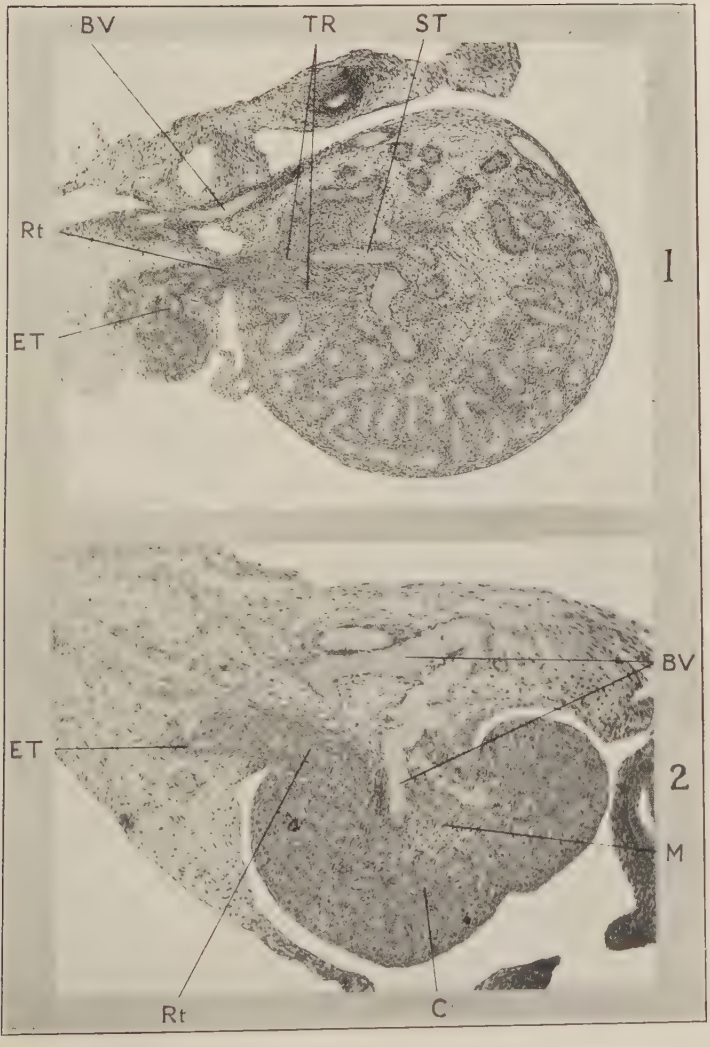
The structure and functions of the ovarian follicles and of the corpora lutea in mammals will be dealt with in the two succeeding chapters.

PLATE XIV

Development of the Gonads of the Mouse

1. Section of the testis of an eighteen-day mouse embryo, showing the spermatic tubules (ST) connected by the tubuli recti (TR) and rete (Rt) with the mesonephric tubules of the epididymis (ET). A blood vessel (BV) is seen entering the gonad. × 65. 2. Section of the ovary of an eighteen-day mouse embryo, showing the vascular supply (BV), the rete (Rt), and the epoophoron (ET). The cortex (C) and medulla (M) of the ovary can be distinguished. × 65. (From [29].)

PLATE XIV



The Urogenital Connection.—The urogenital connection in the female is established in a similar manner to that in the male (Pl. XIV, Fig. 2). The rete canals are formed in the same way as in the male and unite the mesonephric tubules of the anterior end of the mesonephros with the extremities of the medullary cords. The portion of the mesonephros concerned in this union is known as the epoophoron, and is homologous to the epididymis in the male. The posterior portion of the mesonephros, homologous to the paradidymis of the male, which does not participate in the urogenital connection, is the paroophoron.

The rete ovarii loses its connection with the mesonephric tubules at an early stage of development, but persists in the region of the hilum as a functionless network of fine tubules, traces of which are recognisable even in the adult ovary.

The Wolffian duct in the female degenerates while the mesonephric tubules of the epoophoron and paroophoron persist in a degenerate condition and can be found embedded close to the ovary in the broad ligament or mesovarium of the adult. The urogenital connection in the female is thus entirely functionless in all vertebrates and is to be regarded as a vestigial male organ.

The Müllerian duct, which forms the oviduct in the female and is vestigial in the male, is formed by the fusion of two or three peritoneal funnels in the anterior end of the urogenital ridge in front of the developing gonad. The tip of the single funnel thus formed grows back beneath the peritoneal epithelium of the ridge, parallel to the Wolffian duct, and opens into the cloaca. The origin and development of the oviduct is thus entirely unconnected with that of the ovary itself.

CHAPTER VIII

THE OVARIAN FOLLICLE

The oocytes in the ovaries of almost all metazoa are surrounded by one or more cellular layers, forming the follicles. Even the Hydra has a simple epithelial layer around the growing egg-cell. Ovarian follicles are universally present in all vertebrates in varying degrees of complexity. The primitive function of the follicle is undoubtedly to convey nutrition to the large and rapidly growing oocyte. The cells of the follicle which perform this primitive function are epithelial in character and are derived in vertebrates from the medullary and cortical cords which are proliferated into the developing gonad during embryonic life. These cells form a simple or compound layer around the growing oocyte and constitute the follicular epithelium. The follicular epithelium is generally surrounded and supported by a connective tissue coat or theca derived from the surrounding stroma. The whole follicle is, therefore, strictly comparable to the spermatic tubule in the male, since it is derived from the same cellular elements, save that it encloses normally only a single germ-cell instead of a large number.

This simple type of follicle is found in the lower vertebrates but becomes increasingly complex in the higher forms, in which it assumes other important functions as well as that of conveying nutritive material to the oocyte.

The oocytes in Amphioxus are surrounded by a few flattened epithelial cells which constitute the follicle. The follicle in Cyclostomes, Fishes, and Amphibians is composed of a single layer of epithelial cells and a thin theca. The follicular epithelium is columnar in some forms in which processes or fibrillæ of the zona pellucida are formed to attach

the egg, when laid, to the substratum. Further description is unnecessary since the follicle in these forms is not of special interest in the present state of our knowledge. Its function appears to be simply that of supplying nutriment to the oocyte and probably of participating in the formation of the zona pellucida.

The follicle in reptiles consists of a single layer of small epithelial cells around each of the small oocytes. It becomes several cells thick as the oocyte increases in size to meet the relatively huge demands for nutrition imposed on it by the growth of the macrolecithal egg. Loyez [146] distinguishes three sizes of cells in the follicular epithelium of lizards and snakes. The smallest of these can divide mitotically and give rise next the oocyte to the intermediate-sized cells which develop into the large pear-shaped cells. The large follicle cells furnish substances for the formation of yolk to the oocyte. These substances, which may be fluid, semi-fluid, or granular, pass through canalicular prolongations of the cells which penetrate through the zona pellucida, into the periphery of the oocyte. The contents of the nucleus and even of the entire cell may pass into the yolk in this way without having undergone any essential modification, or the follicle cell may undergo first a sort of fatty degeneration and pass into the yolk in the form of fine corpuscles. The theca in reptiles consists of a coat of connective tissue fibroblasts surrounding the follicular epithelium.

The follicles in birds resemble those in reptiles in many respects. Each follicle is surrounded by a connective tissue theca, which is better developed around the larger oocytes. It is composed of fibroblasts arranged concentrically around the follicle, and its inner layers are denser than the outer. The theca is richly supplied with blood vessels and lymph lacunæ and contains islets of large, clear cells, the " luteal " or " interstitial " cells of some authors. The outer surface of the follicular epithelium is clearly defined and is probably separated from the surrounding theca by a fine basement membrane. The follicular epithelium around small oocytes consists of a single layer of cubical cells which appear to secrete actively

substances into the oocyte. The Golgi apparatus in these cells divides in two and half of it passes into the periphery of the oocyte [27]. It persists there for some time and apparently contributes to the formation of yolk. This remarkable phenomenon indicates the active part the follicle cells play in the metabolism of the growing oocyte. This process appears to stop when the oocyte has attained a diameter of about 650 μ. The epithelium then becomes thicker and many layered owing to the active mitoses of its constituent cells. Most of the cells remain unchanged, but some differentiate and acquire a strong affinity for stains. These cells stretch from the periphery of the oocyte to the basement membrane surrounding the follicular epithelium, although the other cells have become arranged in several layers. They act possibly as supporting cells, but finally undergo a kind of colloidal degeneration. During the subsequent growth of the oocyte the various membranes become stretched and, in consequence, thinned. This results finally in the epithelium becoming once more a single layer of flattened cells as in the young oocyte.

The cells of the follicular epithelium have been shown by Fell [78] to form a vacuolated tissue in the cavities of atretic and discharged follicles. Some of the thecal connective tissue cells also play a part. The tissue becomes compressed by the contraction of the follicle and its cells undergo fatty degeneration. The resulting structure has nothing in common with the corpus luteum of mammals, although some authors have attempted to show a resemblance.

The highly specialised character of the follicular epithelium in Sauropsida has been developed apparently in response to the nutritive demands of their large ova.

The follicles in the Monotremes resemble those in the Sauropsida in that they are filled completely by the large oocytes and do not contain the fluid-filled cavities or antra which are characteristic of the Graafian follicles of other mammals. Hill and Gatenby [111] have described the structure of the follicles in detail. Small oocytes up to 0·5 mm. in diameter are surrounded by a single-layered epithelium of flattened cells and a thin thecal coat of concentrically arranged stromal cells.

During the subsequent growth of the oocyte the primitive theca differentiates into an inner cellular and an outer fibrous zone. The outer zone, which is the thicker, consists chiefly of collagen fibres and constitutes the theca externa. The inner zone is composed of oblong cells, compactly arranged in

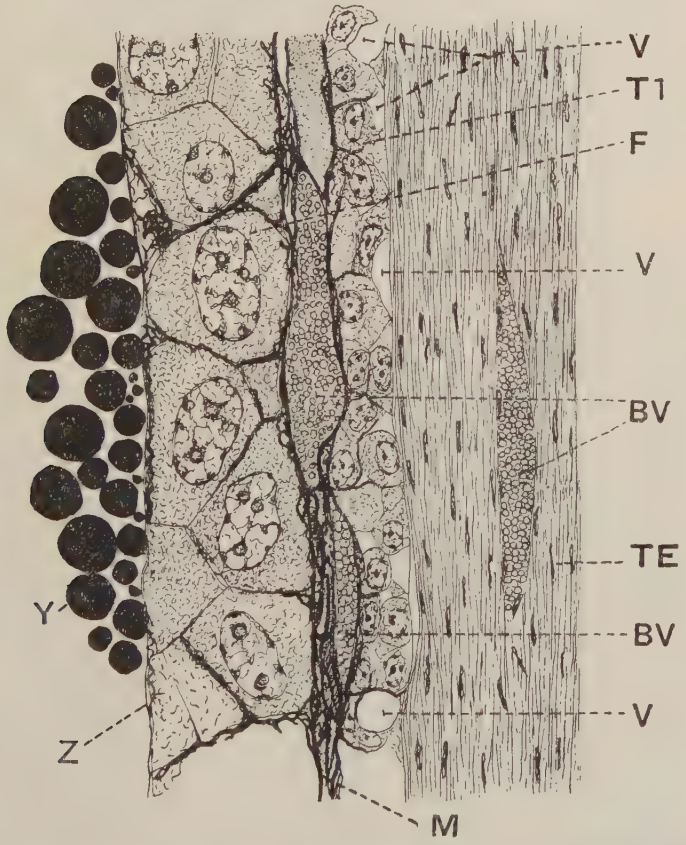


FIG. 10.—Part of the wall of a full-grown follicle of the Platypus (Ornithorhynchus). BV, blood vessels; F, cell of follicular epithelium; M, membrana propria; TE, theca externa; TI, theca interna; V, vacuoles in theca interna; Y, yolk in periphery of oocyte; Z, zona pellucida. × 375. (From Hill and Gatenby[111].)

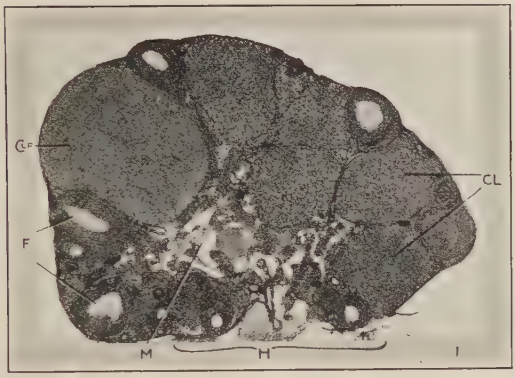
several layers, and a few scattered fibroblasts. The oblong cells form the theca interna, which is characteristic of mammals.

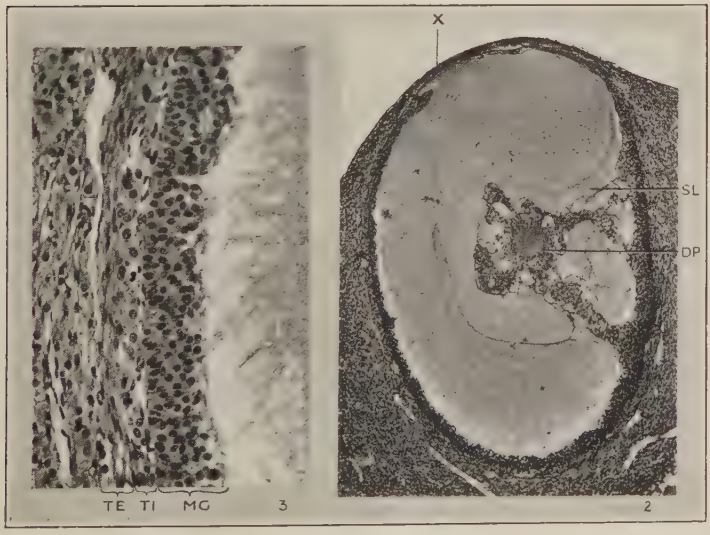
PLATE XV

The Adult Mammalian Ovary

1. Ovary of young adult mouse, showing corpora lutea (CL) and Graafian follicles (F). The large corpus luteum (Clp.) is that of the pregnancy during which the animal was killed. The medulla (M) and the region of the hilum (H) are shown. × 25. 2. Mature Graafian follicle of the rabbit about to rupture. The wall of the follicle and the overlying tissues have become very thin at the point (X) where rupture will take place, and there is some extravasation of blood. The discus proligerus (DP) with the oocyte in it has become partly separate from the wall of the follicle in preparation for ovulation. The thick secondary liquor folliculi is coagulated in a cloud (SL) around the discus proligerus. × 32. 3. Part of the wall of the follicle shown in Fig. 2 at a higher magnification, showing the membrana granulosa (MG), the theca interna (TI), and the theca externa (TE). × 150. (Fig. 2 from Parkes [75].)

PLATE XV





The fibroblasts in this inner zone give rise to a fenestrated connective tissue membrane, the membrana propria, which separates the theca interna from the follicular epithelium and probably invests the cells of the latter. During the later growth of the oocyte the follicular epithelium and the theca interna increase and attain their maximum development shortly before ovulation. The follicular epithelium of the mature follicle (Fig. 10) is composed of plump glandular-looking cells 22 μ by 15 μ in size. These cells form a layer about two cells thick, though an occasional cell may extend right across it.

The theca interna of the mature follicle consists of a discontinuous layer of small cells. It varies from one to five cells in thickness and may be quite absent in places. The cells of the theca interna are oval or oblong in shape and are much smaller than those of the follicular epithelium. They frequently exhibit a vacuolated appearance suggestive of the presence of fat or lipoid droplets in them. Numerous capillaries are present in and between the theca interna and membrana propria.

It is a significant fact that Monotremes differ from Sauropsida in possessing a differentiated theca externa, and in exhibiting a maximum development of the follicular wall at the time of ovulation. These phenomena are to be regarded as preparatory to the formation of the corpus luteum and indicate that the follicle has assumed an endocrine function as well as that of supplying nutriment to the oocyte.

The follicles of Marsupials and placental mammals resemble each other in essential respects and will be considered together. The mature follicles in both contain a large fluid-filled cavity or antrum (Pl. XV). The term "Graafian follicle" should be confined to follicles with this distinctive characteristic.

The Graafian follicle of the mouse is a convenient form to take as a type since our knowledge of its structure and function is specially complete [30].

The smallest or primordial follicles in the mouse are composed of only a few flattened epithelial cells surrounding the oocyte. They measure only about 16·5 μ in diameter, while the enclosed oocyte is approximately 13 μ in diameter.

The follicular epithelium soon becomes cubical, then passes through a double-layered condition to become three-layered by the time the oocyte has attained its full diameter of about 70 μ. The theca has become a definite concentric zone of connective tissue in the meantime. A few glandular cells appear in its inner layers when the follicular epithelium is passing from the double- to the triple-layered condition. These cells increase in number, apparently differentiating from the connective tissue cells of the primitive theca, and form a definite theca interna around the follicle between the fibrous theca externa and the follicular epithelium or membrana granulosa as it is now termed. The cells of the theca interna are almost as large as those of the membrana granulosa, not markedly smaller as in Monotremes. The membrana propria is represented by a slight fibrous reticulum between the membrana granulosa and the theca interna. Shortly after the theca interna has differentiated and the oocyte has attained its full size the antrum is formed. It appears at first as an irregular fluid-filled cleft in the middle of the membrana granulosa at one side of the oocyte. This cleft enlarges as the follicle grows and forms first a crescentic and finally a spherical cavity occupying the major part of the follicle. It is distended by the fluid content or primary liquor folliculi which is rich in colloids. The oocyte, surrounded by a zone of cells, termed the discus proligerus, projects into the antrum on one side. The discus proligerus is composed of cells of the membrana granulosa and is loosely attached to the layer surrounding the antrum. The cells of the discus proligerus become modified and elongated in a radial direction, forming a zona radiata, around the oocyte in follicles approaching maturity. Sections of fixed material at this stage exhibit a thick glairy coagulum which is termed the secondary liquor folliculi in the antrum in the neighbourhood of the oocyte. The antrum attains a maximum size of about 430 μ in diameter. The surrounding layer of the membrana granulosa is about 60 μ thick, and mitosis, which was active during the growth of the follicle, stops at this stage. The surface of the follicle approaches the periphery of the ovary, owing to its growth and possibly to an actual

migration. At the same time the theca interna becomes thin over this peripheral area although it is 30 to 40 μ thick on the other side of the follicle. The antrum is consequently only separated from the body cavity by the peritoneal or germinal epithelium covering the ovary and by a few layers of connective tissue composing the tunica albuginea and theca externa which have become thin at this point. Rupture of the follicle then results in the expulsion of the oocyte, surrounded by the zona radiata, which has become separated from the wall of the follicle. The liquor folliculi escapes at the same time and the follicle collapses. The ruptured follicle continues for a brief time to secrete fluid, the tertiary liquor folliculi, with which it becomes partially distended. Simultaneously, the preliminary changes resulting, as will be seen in the next chapter, in the formation of the corpus luteum, take place. It is apparent, from this account, that the main increase in the size of the follicle is due to the distention of the antrum. This fact is even more apparent in larger mammals in which the mature follicle is many times larger. The ripe follicle attains a diameter of 1·5 cm. in the cow, for instance. The structure of the follicle does not differ essentially in different mammals but varies considerably in minor points. The theca interna cells in the human follicle, unlike those in the mouse, are much larger than those of the membrana granulosa. Some forms such as man, the cat, rabbit, etc., exhibit curious structures in the membrana granulosa known as the bodies of Call and Exener. These consist of occasional scattered groups of cells of the membrana granulosa arranged in a stellate manner around a small central cavity containing liquor folliculi. The exact significance of these is doubtful.

Anovular follicles occur in small numbers in many mammalian ovaries, especially in old individuals. They arise probably in the majority of cases from small follicles in which the oocyte has degenerated, but may develop in immature animals from epithelial cells without the participation of an oocyte. Anovular follicles never attain large dimensions, probably owing to the fact that no antrum is formed within them. This interesting fact suggests that in a normal follicle the liquor folliculi and the

antrum are formed as a reaction to the presence of the oocyte. Anovular follicles in section bear a striking resemblance to sterile spermatic tubules such as occur in undescended testes.

Polyovular follicles also occur in many mammals usually in small numbers. They may contain two or more oocytes and exhibit no other abnormalities. There is no reason to suppose that these oocytes do not ovulate and develop in a normal manner in many cases.

The Primitive Function of the Follicle.—The primitive function of the follicle is to convey nutrition from the circulation to the growing oocyte. This function reaches its climax in the Sauropsida, in which the follicular epithelium around the large-yolked oocytes is very highly specialised.

The follicle in vertebrates may also play an important part in the formation of the zona pellucida, although some authorities maintain that this membrane is secreted entirely by the oocyte. Thing[217], working on reptiles, considers that this membrane is formed entirely by the cells of the follicle, an opinion which the majority of workers appears to hold in regard also to the mammalia.

The follicle in mammals assumes, as well as these, other functions of major importance. The Monotremes resemble the Sauropsida in producing macro-lecithal eggs. Consequently a well-developed follicular epithelium is required to meet the heavy demand for nutrition. The follicle, however, differs from that of the Sauropsida in the presence of a differentiated theca interna. The part which the theca interna plays in the activity of the follicle before ovulation is obscure. It takes a prominent part, however, in the formation of the corpus luteum after ovulation. The appearance of these two structures in the ovaries of Monotremes and not in lower forms is, in itself, highly significant and suggests that the chief function of the theca interna is its participation in the formation of the corpus luteum.

The Function of the Graafian Follicle.—The function of the Graafian follicle of higher mammals is far more complicated and demands much further investigation, although our knowledge is already considerable. The nutrition of the

egg in this case is definitely only one of several functions. This conclusion is evident when we examine the relative growth curve of the oocyte and the follicle. It is then apparent that the major part of the growth of the follicle is effected after the oocyte has attained its maximum size, when its nutritive requirements presumably have been much reduced. The oocyte in the mouse attains its maximum size of 70 μ in diameter when the follicle is approximately 125 μ in diameter, or less than $\frac{1}{4}$ its diameter when about to ovulate. The subsequent growth of the follicle in the mouse is not correlated with any growth of the oocyte. The relative growth curve is shown graphically in Fig. 11. The theca interna is scarcely

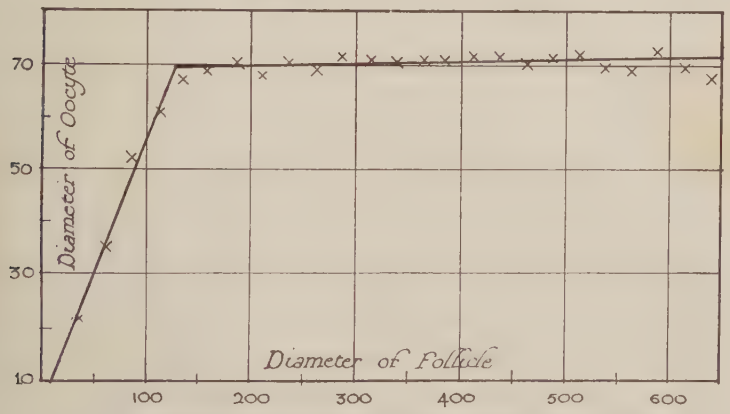


FIG. 11.—Graphical representation of the growth of the oocyte in the mouse plotted against that of the follicle. It is seen that the later growth of the follicle is not correlated with any corresponding growth in the oocyte. (From [30].)

differentiated, being only represented by a few cells, scattered in the theca, and no signs of an antrum are present until after the growth of the oocyte has been completed. It may be assumed, therefore, that these structures are not concerned with the nutrition of the oocyte but serve some other function.

It is well known that the maturation and rupture of the Graafian follicles is strictly correlated with the œstrous cycle and takes place in most mammals during or immediately before or after the period of œstrus or " heat." This time correlation

has led to a long and fruitful series of inquiries into the physiological relation of follicular maturation and œstrus.

The more or less gradual growth of the follicle culminating in ovulation and the simultaneous appearance of œstrus, repeated rhythmically, naturally led the older observers to conclude that the maturation of the follicle produced the œstrous period. Allen, Doisy, and their co-workers [6, 7] were the first to make a great advance on this conception. They made extracts of the liquor folliculi removed from the large follicles of cow and pig ovaries with alcohol-ether-acetone and obtained an active substance which, when injected into ovariotomised rats, produced the œstrous symptoms in the vagina and uterus. They obtained active extracts from the residual ovarian tissue also, but put this down to the incomplete removal of the follicular fluid. They considered the liquor folliculi to be the chief source of the hormone and consequently it was named "folliculine." This work appeared to demonstrate that the follicle elaborated this hormone and that it was liberated at ovulation by the rupture of the follicle, thus producing œstrus.

Many subsequent workers have confirmed Allen and Doisy's discovery of the œstrus hormone and several, especially Parkes and Bellerby [182], have noted its presence in considerable quantities in ovaries from which the large follicles and corpora lutea have been removed, in hollow corpora lutea, and in placentæ as well as in the liquor folliculi of the cow, pig, horse, sheep, and man.

The occurrence of the œstrous hormone in considerable quantities in placentæ appears to show that it is formed during pregnancy. The storage of the hormone in the placenta takes it out of the circulation and may be necessary to prevent the pregnant female coming into œstrus. It may also be an adaptation to prevent the hormone reaching the fœtal circulation and affecting prematurely the genitalia of the developing embryos. This hormone has also been extracted from female urine, and recently by Fee, Marrian, and Parkes [75], it has been obtained in small quantities in human urine from the male. This observation appears to show that the hormone is formed in small

quantities, although it is not known to have any function, in the male. The fact that it could be obtained from residual ovarian tissue as well as from the liquor folliculi cast some doubt on the theory that it was entirely elaborated by the follicle. This doubt was soon amply justified.

The ovaries of mice can be sterilised readily by X-rays. It was found that mice in which all the follicles without exception had been destroyed in this manner continued to exhibit an œstrous cycle similar to that of the normal unmated female [36,37,38]. Further, young mice sterilised by X-rays before puberty came into œstrus at the normal time and subsequently exhibited a regular cycle. These experiments showed clearly that the follicle was not essential for œstrus production and that ovaries entirely devoid of follicles, and consequently of corpora lutea also, elaborate the œstrus-producing hormone in a normal manner. It is clear, therefore, that the œstrous rhythm is not regulated by the periodic maturation of the follicles, and that neither Graafian follicles nor corpora lutea are the essential source of the hormone. This conclusion is confirmed by the fact that several workers have observed that double ovariotomy may be followed within a couple of days by œstrus, although the œstrous cycle stops subsequently. Oestrus may appear in the mouse up to forty-eight hours after double ovariotomy. The ovaries removed from the animals which exhibited œstrus subsequently were examined, and some were found to contain only comparatively small follicles and none approaching the mature size.

These results show clearly that the follicle is not responsible for œstrus production and that the œstrus-producing hormone is not essentially elaborated by them. The hormone has consequently been renamed " œstrin " (Parkes and Bellerby [182]), a term which is now generally accepted in preference to " folliculine." The most recent methods of extraction and assay of œstrin are described by Marrian and Parkes [151].

Since œstrus is not caused by follicular maturation, we are confronted with a new problem : what causes the periodic recurrence of œstrus and ovulation and how are these phenomena synchronised ? It is necessary in attacking this problem

to examine the time relations of œstrus stimulation and of follicular growth.

The fact that œstrus may occur in the mouse up to forty-eight hours after double ovariotomy admits of no other interpretation than that the œstrus stimulus is effected at least forty-eight hours prior to the appearance of œstrus and subsequently proceeds independently of the ovary. This deduction is corroborated by the fact that œstrin injected into the ovariotomised mouse produces œstrus about forty-eight hours afterwards. It is therefore clear that œstrin effects the œstrus stimulation about forty-eight hours before the resulting œstrous period begins. Having arrived at this result we will proceed to examine the time relations of the growth and maturation of the follicles. It has been shown that the mature follicle in the mouse measures on an average approximately 550 μ in diameter, and that ovulation takes place about the time of the onset of the œstrous period [34]. Two days prior to œstrus, at the time when the œstrus stimulation is effected, the follicles about to mature measure on an average about 400 μ in diameter, and immediately after an œstrous period the largest follicles measure about 380 μ in diameter. Follicles of all sizes up to 380 μ in diameter occur plentifully in any adult mouse ovary. It may be concluded that the growth of the follicles is comparatively slow until they attain a diameter of about 400 μ ; then a rapid period of growth begins at the time when the œstrus stimulus is effected. The growth curve of the follicles during the œstrous cycle prior to ovulation is shown in the accompanying graph (Fig. 12) correlated with the stages of the cycle. The hatched columns indicate the time of ovulation and the dotted column the time of œstrus stimulation. The ovaries employed were those removed by double ovariotomy from animals which subsequently came into œstrus a known time afterwards or which had been in œstrus a known time previously. The sizes of the follicles are given as volumes in thousands of cubic μ.

This graph shows clearly that the follicles enter on a final period of very rapid growth at the exact time when the œstrus stimulation is known to be effected. This correlation

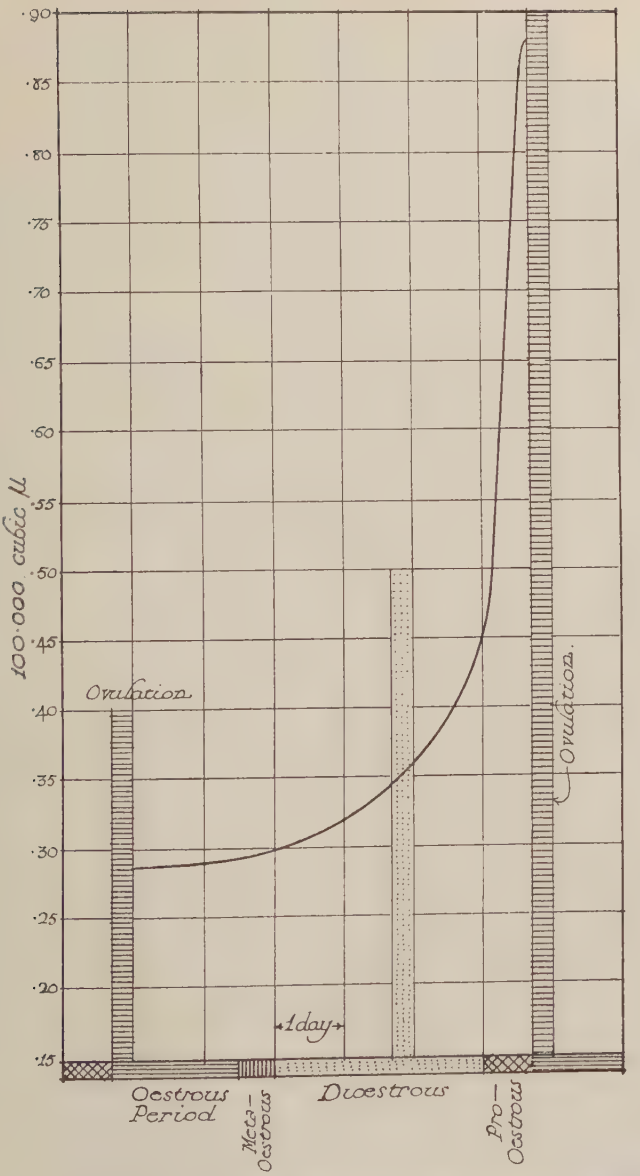


FIG. 12.—Graphical representation of the growth of the larger follicles between two œstrous periods. It can be seen that most of the growth takes place during the two days immediately preceding œstrus. (From [34].)

suggests that either (*a*) œstrin stimulates the maturation of the follicles as well as the œstrous period, or (*b*) some other factor stimulates simultaneously the maturation of the follicles and the release of the œstrin which brings about œstrus.

The former hypothesis has been tested directly and has not been satisfactorily confirmed. Frank, Kingery, and Gustavson [82] claim to have caused ovulation in the immature rat by œstrin injection, but, on the contrary, a number of other workers have obtained negative results.

Recent work appears to establish definitely the second hypothesis. Smith and Engle [209], and also Zondek and Aschheim [252, 253], have found that by injecting the substance of the anterior pituitary in the form of aqueous suspensions or extracts into mice they were able to produce ovulation and œstrus. The injection in adult animals stimulates all the larger Graafian follicles to undergo rapid growth and maturation. Ovulation follows and may result in the liberation of dozens of ova at the same time, and in the production from the ruptured follicles of a large number of corpora lutea. The effect on the accessory organs is to produce œstrus within three days. Mating at this time may result in the production of an abnormally large number of embryos, many of which may become implanted. Similar results may be produced in the immature fortnight-old mouse and are then even more striking. These pituitary injections produce absolutely no effect on ovariotomised animals; it is therefore clear that they act through the ovary and produce œstrus by effecting the release of œstrin. The simultaneous maturation of the follicles and release of œstrin in the ovary therefore depend on a third factor resident in the anterior pituitary. This factor is apparently of the nature of a hormone, and is that which governs the constant number of follicles which ovulate at each period. This interpretation is due to Engle [71, 72], who found that compensatory hypertrophy in partially ovariotomised animals was accelerated by injection of anterior pituitary. Some support for this view is also provided by experiments on mice in which one ovary was completely sterilised by X-rays [35]. The normal ovaries in these animals exhibited complete compensatory

hypertrophy, although the X-rayed ovaries are known to be capable of maintaining the œstrous cycle. The hypertrophied ovaries not only weighed on an average as much as a pair of normal ovaries, but the litters produced by them were almost as large as those of normal animals. It is therefore clear that compensatory hypertrophy of the ovary is the result of an increase in the number of follicles maturing and a consequent increase in the number of corpora lutea formed at each œstrous period, and that this increase, in the case of the absence or sterility of one ovary, is approximately 100 per cent. These experiments support Engle's in so far as they indicate that the factor controlling this constant number of maturing follicles is situated outside the ovaries.

It follows from these results that the periodic mechanism governing the cyclic activity of the female reproductive organs is situated in the anterior pituitary and not in the ovary as was believed previously. The ultimate problem of the nature of this mechanism remains, however, unsolved since the anterior pituitary does not exhibit any visible periodic mechanism.

The problem of the morphological and physiological significance of the mammalian Graafian follicle remains to be solved. The work described in this chapter offers no explanations, although it destroys those that have been generally accepted heretofore. At the time when the nutrition of the oocyte is virtually completed its chief characteristics, the theca interna and the antrum containing liquor folliculi, have scarcely begun to develop. The obvious explanation that the Graafian follicle has been evolved as a cyclic endocrine organ to control the œstrous rhythm is also disproved by the work described above. There are, however, several clues which may lead to the solution of this fascinating problem. First of all the Graafian follicle is only found in Marsupials and Placentals, all of which have microlecithal eggs, and is not developed in Monotremes which have macrolecithal eggs. We may conclude that in mammals the evolution of the Graafian follicle is correlated with that of the microlecithal egg. Its size, on the contrary, bears some relation to the bulk of the animal, while that of the egg does not. Finally, its structural

characteristics are developed immediately before œstrus and ovulation, at which time we may suspect its characteristic function to be performed. Two explanations of the function of the Graafian follicle then present themselves : (1) that it is a mechanical adaptation to effect ovulation ; (2) that it is adapted to supply a large area of follicular wall so as to effect the rapid formation and growth of the corpus luteum after ovulation. In reference to the first it is obvious that the small microlecithal egg of the higher mammals could not easily burst through the ovarian cortex by the pressure exerted by its own growth, whereas the large, rapidly growing macrolecithal eggs of Sauropsidans and Monotremes would find no difficulty in doing so. The large and rapidly distending Graafian follicle would correct this deficiency and would be capable of bursting through the superficial tissues by reason of its own internal pressure, and so effecting ovulation. This hypothesis might admit of direct test by measuring the hydrostatic pressure in the antrum of maturing follicles. The second hypothesis is also obvious, when it is remembered that the rapid attainment of functional activity on the part of the corpus luteum is essential for the preparation of the uterine mucosa for implantation and for the maintenance of pregnancy. The Monotreme follicle affords a sufficiently large cavity for the formation of the corpus luteum, owing to the large size of the oocyte, without any augmentation by the development of an antrum. Both these explanations of the function of the Graafian follicle appear probable and both may be true.

It must be admitted, however, that the function of the follicle, even in mammals, is by no means fully understood and affords wide scope for further research.

CHAPTER IX

THE CORPUS LUTEUM

The Formation of the Corpus Luteum.—The wall of the ruptured ovarian follicle collapses after the escape of the oocyte and retrogresses sooner or later. The retrogression of the follicular remains begins, especially in Elasmobranchs and Sauropsidans, as a process of fatty or lipoidal infiltration of the cells accompanied by the ingrowth of connective tissue cells from the theca and the formation of an orange pigment, probably from extravasated blood. This phase results in the formation of a more or less solid nodule of tissue, in the cavity of the former follicle, which gradually retrogresses and finally disappears. Fell[78] considers that clear islet cells found in the stroma of the bird ovary may be formed in part from such degenerating follicles.

The ruptured follicle in mammals does not degenerate in this manner but transforms into an important endocrine organ, the corpus luteum. It has been claimed that the tissue in the discharged follicles of Elasmobranchs and Sauropsidans is comparable to the corpus luteum of the Mammalia, but there is no satisfactory reason for accepting this view, since the former is a degenerative structure and the latter a highly organised gland with a characteristic structure common to all mammals, even to the Monotremes.

The fact that the corpus luteum is found in all mammals but not in any other group suggests that it has been evolved to meet the special requirements of gestation and lactation. The physiological findings entirely support this supposition and show that it is an endocrine gland essentially concerned with the hormonal control of these processes.

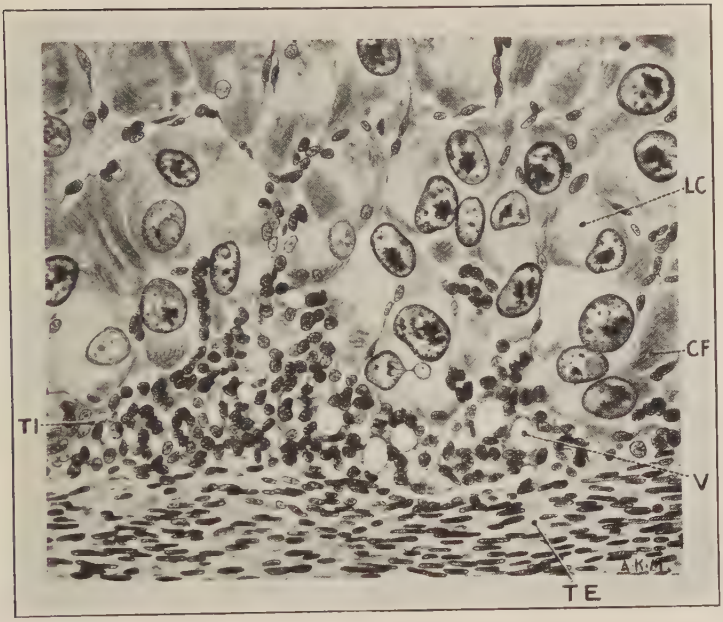
Little was known of the structure of the corpus luteum of Monotremes until the recent publication of a paper by Hill

PLATE XVI

The Corpus Luteum of the Platypus (Ornithorhynchus)

1. Portion of the periphery of the corpus luteum, showing the theca externa (TE), the theca interna (TI), forming a layer of variable thickness which is syncytial in character and contains large vacuoles (V), and the luteal cells (LC) with rod-shaped mitochondria (CF) in them. × 300. 2. Section through a whole corpus luteum. The core (CC) is formed of the remains of the blood-clot, formed in the ruptured follicle, and of connective tissue. The theca externa (TE) has given rise to ingrowths into the corpus luteum, along which the theca interna cells are situated. A plug (OP) of luteal tissue occupies the site of the rupture of the follicle. LC, luteal cells; OO, oocyte; LS, lymph sinuses in the ovarian stroma. × 23. (From Hill and Gatenby[111].)

PLATE XVI



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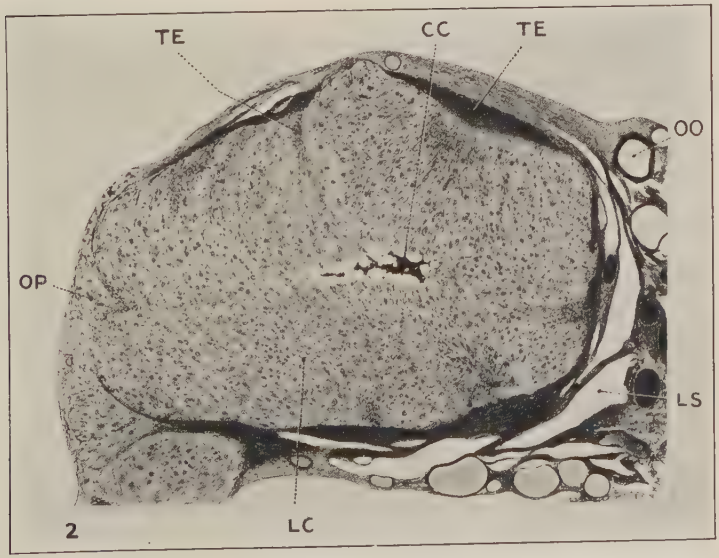

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and Gatenby [111] which removed this deficiency and has done much to elucidate the more difficult problem of the formation of the corpora lutea of higher mammals. The importance of this work is due to the fact that the distinction between the three layers of the wall of the full-grown follicle (Fig. 10) is so definite and clear-cut that it is a relatively simple matter to follow their derivatives during the formation of the corpus luteum.

Ovulation results immediately in the collapse and contraction of the ruptured follicle, accompanied by folding of its wall. The follicular epithelium protrudes around the rupture, and the cavity of the follicle remains partially filled with extravasated blood and cells detached from the wall. The formation within the ruptured follicle of the corpus luteum (Pl. XVI) is rapid and is contributed to by all three layers of the follicular wall, the ingrowths of which soon fill the follicular cavity.

The theca externa grows in actively at a number of points as fibrous trabeculæ which form the connective tissue framework of the corpus luteum (Fig. 13). Blood and lymph capillaries are conveyed into the corpus luteum chiefly along these trabeculæ, and the theca interna cells also grow in along them. The theca interna cells multiply by mitosis and form syncytial-like masses and groups. The follicular epithelium does not appear to exhibit any mitoses but grows by the hypertrophy of the individual cells. These cells increase to almost three times their original diameter and give rise exclusively to the "luteal" cells. These luteal cells are by far the largest elements in the corpus luteum and are the main factors in effecting its formation. They are always easily distinguishable from the other elements and appear to attain their greatest development rather later than the theca interna elements. The fully formed corpus luteum attains a size of about 4·5 mm. by 3·5 mm. Retrogression begins shortly before the eggs are laid and results in the degeneration of the luteal and theca interna cells accompanied by an increase in the amount of connective tissue and the appearance of numerous leucocytes.

The cytological character of the luteal and theca interna cells shows that both are glandular in character and probably subserve secretory functions in the corpus luteum. The two types of cells, however, are widely different in character and the theca interna cells appear to attain their maximum activity before the luteal cells. This suggests that the corpus luteum elaborates two distinct secretions and may have a dual endocrine function.

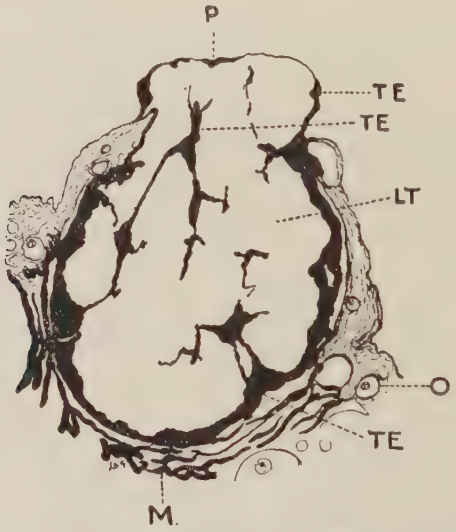


FIG. 13.—Section of a corpus luteum of the Platypus (Ornithorhynchus) showing the theca externa and its trabecular ingrowths (TE) in black. LT, luteal tissue ; M, medulla of ovary ; O, oocyte in ovarian cortex ; P, plug of the corpus luteum. × 7·7. (From Hill and Gatenby[111].)

The origin of the glandular cells composing the corpora lutea of Marsupial and Placental Mammals has excited much controversy. It has been maintained that they originate exclusively from the membrana granulosa, or from the theca interna, or again that both participate. This confusion is due to the fact that there is no clear size-difference between the theca interna and granulosa elements, such as exists in Monotremes. It is consequently extremely difficult to distinguish and follow these elements during the formation of the corpus

luteum. Recent work, based on Hill and Gatenby's findings, has elucidated the problem and has shown that the mode of formation of the corpus luteum in higher mammals is essentially the same as in the Monotremes.

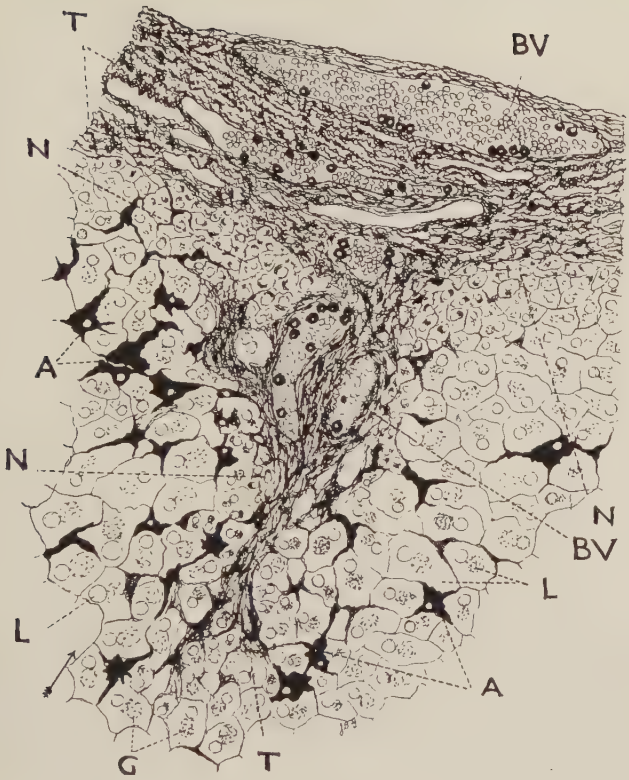
The theca interna cells of the human Graafian follicle are considerably larger than those of the membrana granulosa. They are stellate in shape and have small eccentric nuclei. Gatenby [85] has shown that they can be blackened by treatment with silver nitrate. The cells of the membrana granulosa are more regular in outline and have relatively larger nuclei. They do not blacken with silver. During the formation of the corpus luteum the cells of the follicular wall grow in and become organised in a manner similar to that described in the Monotremes. The membrana granulosa cells hypertrophy and give rise to the luteal cells, which equal in size the theca interna elements. Consequently it is almost impossible to distinguish these elements in the corpus luteum by the ordinary methods. Gatenby, however, showed that the theca interna cells retained their affinity for silver, and that they remained distinct in the corpus luteum (Pl. XVII). They were scattered among the luteal cells and were not distinctly larger than in the mature follicle. The luteal cells contain a greatly enlarged Golgi apparatus and the lutein granules, which impart the characteristic yellow or red colour to the mammalian corpus luteum. The theca interna elements forming the argentophil reticulum may be distinguished as " para-lutein " cells. Solomons and Gatenby [210] demonstrated a similar reticulum of argentophil stellate paralutein cells in the corpus luteum of the pig. The author succeeded in staining similar cells with iron-hæmatoxylin after Regaud's fixation in the ovary of the rabbit (Fig. 14). These recent results appear to warrant the supposition that further work with suitable techniques will show that both theca interna and granulosa cells take part in the formation of the corpus luteum and retain their individuality, as para-lutein and luteal cells respectively, in other mammals. Since both types of cell appear glandular in character these histological findings suggest that the corpus luteum possesses a dual secretory function. These conclusions are in accord

PLATE XVII

The Corpus Luteum of Man

1. Part of the periphery of a human corpus luteum, showing an ingrowth of the theca externa (T). The cells of the membrana granulosa (N) nearest the walls have not yet transformed into luteal cells, while those further out (L) have done so. The enlarged Golgi apparatus of the luteal cells is shown (G). The stellate para-lutein cells (A), which originate from the theca interna have been blackened by the silver. BV, blood vessels. × 316. (From Gatenby [85].) 2. Part of the wall of a mature human Graafian follicle, showing the theca externa (T), the theca interna (TI) of stellate cells which have blackened with the silver, the membrana propria (M), and the membrana granulosa (G). × 454. (After Solomons and Gatenby [210].)

PLATE XVII



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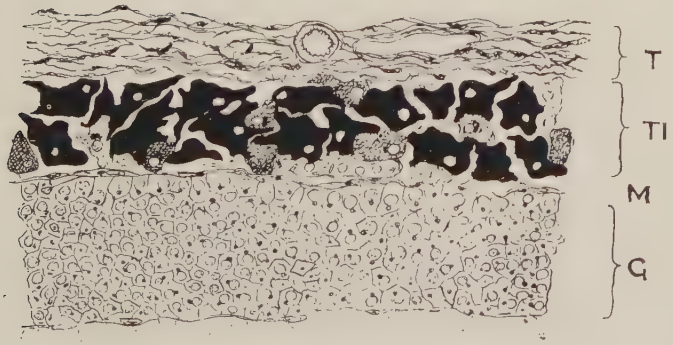

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with the significant fact, already referred to, that the theca interna is found only in the follicles approaching maturity of mammals. It is, presumably, an adaptation for the formation of the corpus luteum.

The ruptured Graafian follicle often continues to secrete liquor folliculi for a short time. This tertiary liquor partially

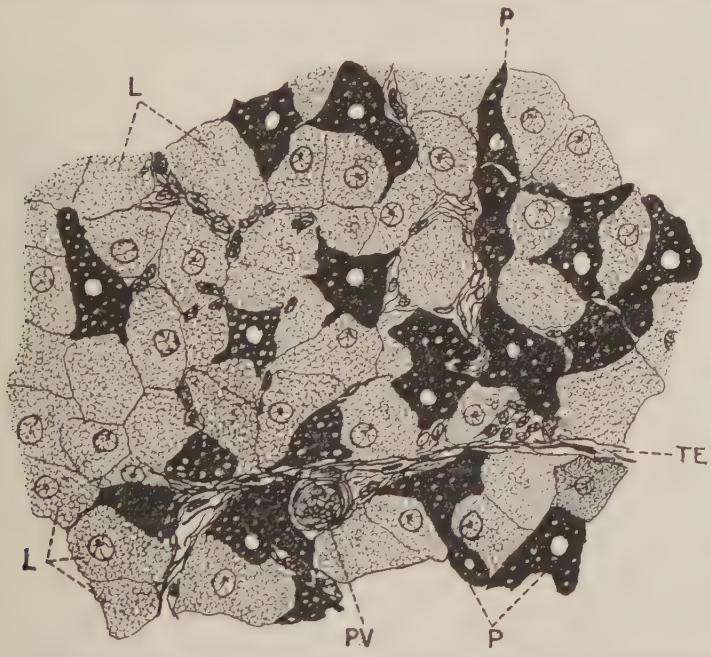


FIG. 14.—Part of the ovary of a rabbit, showing the "interstitial" tissue, derived from degenerate corpora lutea. Black stellate cells (P) are scattered among these (L) derived from the luteal cells. Connective tissue trabeculæ (TE) with blood vessels (PV) can be seen. × 440.

distends the ruptured follicle and forms a fluid-filled cavity which may or may not persist in the centre of the developing corpus luteum.

The Stimulus causing Luteinisation.—The stimulus which brings about the formation of the corpora lutea, like that which results in ovulation, appears to depend on the action of the anterior pituitary. Evans [74] found that sodium hydroxide extracts of the anterior pituitaries of oxen when injected into

rats produced intense luteinisation of the ovaries. All the larger follicles, apparently without ovulating, rapidly transformed into corpora lutea. Further injections enhanced the effect and ovaries could be produced containing dozens of corpora lutea. These changes were correlated with the cessation of the œstrous cycle in non-pregnant animals, owing to the inhibitory action of the corpora lutea. Similar injections into pregnant animals resulted in abnormal lengthening of the gestation period, owing to the stimulated tissue failing to atrophy at the proper time. The luteal tissue resulting from these injections also produced the hypertrophy and sensitivity of the uterus which resulted from the activity of normal corpora lutea. Other workers have confirmed these results.

It is probable, then, that the stimulus for the formation of luteal tissue is provided by a secretion of the anterior pituitary and that the presence of ruptured follicles is not a necessary condition. It is remarkable that aqueous extracts of the anterior pituitary produce ovulation (as shown in the last chapter), while sodium hydroxide extracts produce luteinisation without ovulation. The relation of these two extracts is not yet clear, but while it seems probable that two distinct factors are concerned it is possible that there is only a single factor in different concentrations. In other words, it may result in ovulation when dilute but produce immediate luteinisation when concentrated. It must be remembered that while ovulation takes place spontaneously at œstrus in the mouse, it does not occur and œstrus is prolonged in some animals, such as the rabbit and the ferret, until copulation has taken place.

Different Types of Corpora Lutea.—The fate of the corpus luteum after it is formed depends on whether copulation, pregnancy, or lactation follows ovulation. The corpus luteum in the unmated female normally enters on retrogression shortly before the next ovulation, and its duration in the ovary consequently depends on the length of the œstrous cycle in any given species of mammal. Copulation has the effect in some animals of lengthening the interval before the next œstrous period occurs. The unmated cycle in the mouse, for instance, was found to occupy on an average 5·7 days, whereas the cycle,

after copulation with a sterile buck, was found to last eleven days. This lengthened cycle resembles the early stages of pregnancy in several respects, and has consequently been named " pseudo-pregnancy " by Hill and O'Donoghue [112]. During pseudo-pregnancy the corpus luteum persists for a longer time than in the case of the unmated mouse. It persists still longer in pregnancy, which lasts nineteen days in the mouse, and only retrogresses shortly before parturition. During pseudo-pregnancy and pregnancy the corpus luteum undergoes an increased development and performs important secretory functions. It grows a little by the enlargement of the luteal cells and the lipoidal granules in them increase in size. The corpora lutea of pregnancy are therefore identical with those of ovulation and pseudo-pregnancy at first, but can be distinguished during the latter half of pregnancy. (For details refer to Long and Evans [145].)

The corpora lutea formed at the post-partum œstrous period, which immediately follows parturition in the mouse and rat, persist during lactation when it occurs. The normal period of lactation in the mouse lasts from twenty-one to thirty days. Long and Evans [145] state that the corpora lutea of lactation in the rat differ from the other three types in having smaller lipoid granules in the cells. Further work on the detailed cytology, especially on the cytoplasmic inclusions, of the lutein and para-lutein cells during these four distinct functional phases of ovulation, pseudo-pregnancy, pregnancy and lactation, is required and probably would furnish exceedingly valuable results.

Occasionally follicles fail to ovulate, and transform into corpora lutea without rupturing. The resulting corpora lutea atretica, which can be distinguished by the presence in them of the degenerating oocyte, are of fairly frequent occurrence in nature. They do not differ essentially from normal corpora lutea.

Luteal Retrogression.—Sooner or later all corpora lutea retrogress and finally disappear. The early stages of retrogression are marked by vacuolation and other changes in the cells, accompanied by fibrosis and frequently by marked

leucocytosis. Finally the old corpus luteum is reduced to a small fibrous mass, the corpus albicans, in the stroma. It is claimed that in some animals the luteal cells transform into interstitial cells which persist in the ovary. Retrogression is slow, and several generations of corpora lutea can generally be distinguished in any adult ovary. The presence of large follicles or growing corpora lutea in the ovary appears to accelerate retrogression in the corpora lutea, probably owing to the mechanical or physiological conditions resulting from the local overcrowding.

The Effect of the Corpus Luteum on the Œstrous Cycle.—The œstrous cycles of various kinds of mammals differ very widely in character and have proved extremely difficult to compare. Recent work [181a] has thrown light on the cycles of some of the commoner laboratory animals, such as the rat, mouse, rabbit, guinea-pig, etc., and has resulted in a reasonable analysis and comparison of these with each other and with the menstrual cycle in man.

The simplest type of œstrous cycle is found in the unmated rat or mouse. Œstrus in the unmated mouse recurs every five days on an average and is marked by characteristic changes in the uterine and vaginal mucosa, as well as by ovulation and the formation of corpora lutea. This cycle depends on the rhythmic production or liberation of œstrin by the ovary, since it stops if the ovaries are removed. There is reason to believe, however, that this ovarian rhythm is governed by a periodic mechanism situated in the anterior lobe of the pituitary. It is quite independent of the presence of Graafian follicles or corpora lutea in the ovaries, since it continues undisturbed for an indefinite time after these structures have been completely eliminated by X-rays. It is obvious, therefore, that the corpus luteum does not affect the œstrous cycle, nor is it known to have any function in the unmated mouse.

The mated cycle in the mouse is, however, quite different, since œstrus recurs only every twelve days. This has been shown by mating the females with young males which copulate normally but which have been sterilised by vasectomy. The lengthening of the cycle in this case is due to the introduction

of a pseudo-pregnant phase which closely resembles the early stages of pregnancy in the condition of the uterine mucosa and corpora lutea and in the absence of ovulation. This effect appears to result from the stimulation of the uterine cervix by the hard vaginal plug, formed after copulation from the ejaculated secretion of the vesiculæ seminales of the male. It is possible to produce a similar condition in the unmated mouse by artificial stimulation of the uterine cervix during œstrus. Cervical stimulation does not produce this result directly, since it has no effect in ovariotomised animals, but through its effect on the ovary. It appears to act by stimulating the corpus luteum to function and to produce the pseudo-pregnant phase of the cycle. The cycle in mice which have been sterilised by X-rays and therefore contain no functional corpora lutea is not lengthened by mating, but remains the same as in unmated mice. This observation provides a check for the hypothesis that the corpus luteum is instrumental in producing the pseudo-pregnant phase of the cycle. Œstrus in the mated mouse is thus followed by a stage, strictly comparable to the diœstrus of the unmated female, in which the corpora lutea are non-functional, and by a second stage of pseudo-pregnancy, regulated by the secretion of the corpus luteum, which closely resembles the initial stages of pregnancy. This stage terminates in a degeneration or disruption of the hypertrophied uterine mucosa immediately prior to the onset of the succeeding œstrus. This pseudo-pregnant degeneration therefore marks the cessation of function of the corpora lutea and is often accompanied by slight vaginal and uterine bleeding. Moreover, in the light of recent work there can be little doubt that menstruation in man corresponds to this pseudo-pregnant degeneration observed in the mated cycle of the mouse.

The normal unmated cycle in many animals contains a distinct luteal phase corresponding to the pseudo-pregnant condition which only results from copulation in the mouse and rat. These animals, therefore, never exhibit the basic œstrous rhythm, characteristic of the unmated mouse, in an unmasked condition but with a luteal phase always superimposed. The guinea-pig and the dog exhibit this type of

cycle. The rabbit does so also, but it must be remembered that in this species, as in a few others, ovulation and the consequent formation of corpora lutea do not take place until copulation is effected ; the œstrous period being more or less indefinitely prolonged in the unmated animals.

The Function of the Corpus Luteum in Pregnancy.—The initial stages of pregnancy resemble the luteal or pseudo-pregnant phase of the mated cycle in the mouse and rat and the corresponding stages in other animals in all essential respects. The functions of the corpus luteum in both these conditions appear to be identical. They include the inhibition of œstrus, the preparation of the uterus for implantation of the ovum, and the stimulation of the mammary glands to develop.

Inhibition of Œstrus.—The evidence for the inhibitory effect of the corpus luteum on œstrus, in preventing both the œstrous changes in the uterus and vagina and ovulation in the ovary, is considerable. This effect is indicated by the results referred to above on the mouse and rat where mating normally lengthens the cycle by introducing a pseudo-pregnant phase, but fails to do so in animals which have been sterilised by X-rays and consequently have no corpora lutea in the ovaries. Another line of evidence is provided by the observation that the removal of the corpora lutea from the ovaries expedites the appearance of the next œstrous period in the cow (Hammond [101]) and in the guinea-pig (Loeb [144]). The most convincing evidence, however, depends on the preparation of an extract of the corpus luteum which will, on injection, inhibit œstrus. Many have attempted to produce such an extract, but the majority have obtained negative results. A few claim to have produced positive results of which the most striking are those recently described by Parkes and Bellerby [186]. They prepared extracts of the solid corpora lutea of the cow by grinding the tissue with anhydrous sodium sulphate and extracting with ether and acetone. The injection of the emulsified extract temporarily stopped the œstrous cycle in the normal mouse. The injections to be effective were necessarily large but were carefully controlled by injections of equal amounts of inert emulsions. These observations, coupled with

those which will be referred to in the section dealing with the function of the corpora lutea during lactation, warrant the conclusion that the corpus luteum exerts an œstrus-inhibiting action.

Effect on the Uterus.—Regarding the function of the corpus luteum in preparing the uterus for pregnancy it has been noted already that the luteal phase of the cycle in all mammals is accompanied by characteristic changes in the uterine mucosa. The uterus undergoes growth and vascularisation and exhibits hypertrophy of the glands in the mucosa, whether fertilisation has taken place or not. Hill and O'Donoghue [112] first introduced the term "pseudo-pregnancy" for this condition in the non-pregnant Dasyurus. Such changes are absent, of course, from the unmated cycle of the mouse and rat, but occur after copulation in these animals and in the rabbit irrespective of whether pregnancy does or does not ensue. They occur in the dog and guinea-pig without copulation. This condition of the uterus is obviously a preparation for pregnancy and is characterised by a sensitivity of the uterus to irritation which is not exhibited at other times. This sensitivity apparently provides for the formation of the placenta on the stimulation resulting from the implantation of the ovum. Loeb [143] was able to produce in the uterus large blocks of tissue or placentomata, which resembled masses of decidual cells, by cutting the uterus of the guinea-pig when in this condition. It was found that these placentomata were produced only when the injury occurred a definite time after ovulation. Moreover, they were not produced when either both ovaries or all the corpora lutea were removed by operation. Long and Evans [145] were able to produce similar placentomata in the pseudo-pregnant rat by the introduction of a small loop of surgical silk into the uterine lumen, but were unable to produce them at any stage of the unmated cycle. Similar results have been obtained by Parkes [180] in the mouse and by other authors. The results referred to indicate that the corpus luteum produces uterine hypertrophy and sensitivity preparatory to implantation of the ovum and the formation of the placenta. This conclusion is confirmed by a remarkable

series of experiments with the injection of the sodium hydroxide extracts of the anterior pituitary referred to earlier in this chapter. Teel [215] found that the injection of this extract into the unmated rat resulted in uterine sensitivity, as shown by the production of placentomata after artificial irritation, as well as in the inhibition of œstrus. This result was indirect, since it was not obtained in ovariotomised animals. Evidently the pituitary extract stimulated hypertrophy of the luteal tissue, the activity of which produced the sensitivity of the uterus. Long and Evans [145] and Parkes [180] obtained completely confirmatory results in the rat and the mouse.

Effect on the Mammary Glands.—Milk production in the mammary glands is essentially a breakdown process following a prolonged period of growth and development. Such growth and development of the mammary glands normally takes place during pregnancy and pseudo-pregnancy. The growth stage in the pseudo-pregnant rabbit continues for fourteen days and consists of the lateral extension and swelling of the glands. At the end of this period retrogression sets in, but does not result in milk secretion, since the previous development was insufficient. The development of the mammary glands during pregnancy is identical for the first fourteen days, but continues and results in a further extension and thickening. At the end of pregnancy, when the corpora lutea retrogress, the breakdown stage sets in and results in lactation.

The development during pseudo-pregnancy may proceed so far in some animals such as Dasyurus (Hill and O'Donoghue [112]) and occasionally in the dog (Marshall and Halnan [153]) that lactation may actually take place at the end.

The correlation existing between the growth stage of the mammary tissue and the presence of functional corpora lutea, and of the onset of lactation soon after the beginning of luteal retrogression, obviously suggests a functional relation.

Parkes [181], working on this idea, has produced by the injection of the sodium hydroxide extract of the anterior pituitary into unmated rabbits a development of the mammary glands exceeding that found during normal pseudo-pregnancy. This result is due apparently to the action of the luteal tissue

produced in the ovary under the influence of the anterior pituitary extract. It is therefore fairly well established that the development of the mammary glands is stimulated by the endocrine function of the corpora lutea.

The Maintenance of Pregnancy.—These three functions of the corpus luteum—inhibiting œstrus, preparing the uterus for implantation and placentation, and stimulating the mammary glands—are performed both in pseudo-pregnancy and pregnancy. They are, however, continued longer in pregnancy, in the maintenance of which the corpus luteum appears to play an important part.

It has been recognised for a long time that the corpus luteum plays a rôle in maintaining pregnancy, but experiments failed to throw much light on its exact function. The removal of the corpora lutea during pregnancy gave conflicting results, some authors stating that pregnancy continued without them, others that abortion or resorption followed inevitably. It is probable that these results depended largely on the difficulty of removing surgically the corpora lutea and on the various methods employed and possibly also on the species of animal employed. These difficulties have been overcome in the mouse by means of X-rays [35, 177]. This was done by means of sterilising the ovary on one side, thereby removing the power to produce follicles or corpora lutea, without affecting the other ovary. Such animals were able to become pregnant from the untouched ovary and were allowed to do so. It was found that the removal of the non-irradiated ovary invariably resulted in the termination of pregnancy, although the removal of the sterilised ovary had no effect. Since the sterilised ovaries are known to be capable of maintaining the basic œstrous cycle this result must have been due to the absence of corpora lutea in them. It is therefore apparent that corpora lutea are essential for the maintenance of pregnancy in the mouse.

Teel [216] has obtained important results by injections of sodium hydroxide extracts of anterior pituitary into pregnant rats. These injections resulted in the gestation period being prolonged by from two to six days, apparently owing to the continued activity of the luteal tissue produced by the stimula-

tion with anterior pituitary. The pregnancies in these experiments were terminated by the death and consequent expulsion of the embryos.

Another line of evidence is provided by the results of œstrin injection during pregnancy. Parkes and Bellerby [183] have shown that pregnancy in the mouse can be terminated at all stages by the administration of an adequate dose. The amount of the dose required increased during pregnancy to about double. It is suggested that these results are due to functional overriding of the corpora lutea by œstrin. Such a conclusion indicates that the essential function of the corpora lutea in maintaining pregnancy may be the inhibition of œstrus, but much further work is required before a definite conclusion can be formulated.

The Relation between the Corpora Lutea and Parturition.—Since the corpora lutea are essential for maintaining pregnancy it is possible that parturition is brought about by their retrogression. This hypothesis is strengthened by the fact that luteal retrogression sets in shortly before parturition. It is well known, however, that pituitrin causes uterine contraction and accelerates labour. Knaus [133] has obtained interesting results concerning the susceptibility to pituitrin of uterine muscle during pregnancy in the rabbit. He eliminated the possibility of increased length of the muscle fibres due to stretching during pregnancy, by unilateral ovariotomy. This resulted in one cornu of the uterus, which was sterile and consequently unstretched, being available for experiment while the other was pregnant. It was found that the uterine sensitivity to pituitrin varied considerably but was definitely sub-normal during the greater part of pregnancy. The sensitivity increased, however, at the very end of pregnancy. Knaus considers that the corpora lutea reduce the sensitivity of the uterus during pregnancy and thus prevent parturition. The uterine sensitivity then returns to normal when the corpora lutea atrophy and parturition ensues as a result of pituitrin stimulation. This explanation is satisfactory, but needs further confirmation.

The Function of the Corpus Luteum during Lactation.—

Lactation begins immediately after parturition, apparently as a disruptive process following the period of growth under the influence of the corpus luteum. Lactation itself is therefore brought about finally, in the mouse at least, by the degeneration of the corpus luteum of pregnancy. The majority of animals ovulate soon after parturition, and the corpora lutea formed at this time in the rat and the mouse persist during lactation. The œstrous cycle continues during lactation in many animals, unlike the mouse and the rat, and is normal. The presence of these corpora lutea is not, however, essential either for starting or for maintaining lactation. This has been shown by performing double ovariotomy in the mouse immediately after parturition, when lactation proceeds normally.

Our knowledge of both the structure and function of the corpus luteum is still far from complete, and rich rewards are still to be won by their study. The physiology, in particular, of the corpus luteum obviously opens up prospects of far-reaching clinical application, in the treatment of human disorders, which it is legitimate to hope will soon be attained. The history of the corpus luteum from the point of view of pure science is equally interesting. The corpus luteum has been evolved from the degenerate tissue of resorbing follicles into a complex and highly specialised organ of internal secretion concerned with the endocrin control of the œstrous cycle, pregnancy, and lactation. It is, as such, essentially a mammalian organ adapted to control and co-ordinate the elaborate processes of intra-uterine development and milk secretion.

CHAPTER X

HERMAPHRODITISM

HERMAPHRODITISM and intersexuality have been used more or less synonymously to describe every possible combination of male and female structures in one individual. In this book the term *hermaphrodite* will be limited to those cases in which both ovarian and testicular tissue are present in one individual. The term *intersex* will be used, for convenience, for individuals with the gonads of one sex and some or all of the accessory organs and secondary characters of the other sex, although this term is the more general and properly includes hermaphrodites as well.

True hermaphroditism may be conveniently classed under two headings: normal and abnormal. Many invertebrates are normal hermaphrodites, every individual producing both kinds of gametes either at different times or simultaneously; some are even self-fertilising. Normal hermaphroditism is very rare among vertebrates, but a few cases are known in adults. Some other vertebrates, in which the sexes are distinct when adult, pass through a transitory hermaphrodite condition during development. Although these cases of juvenile hermaphroditism are not functional hermaphrodites they appear to be a normal developmental stage and can be considered most conveniently as sex-reversals.

Hermaphrodite individuals of normally diœcious forms occur in all vertebrate phyla. Some species exhibit this abnormality comparatively frequently, while in others it is unknown. These cases of abnormal hermaphroditism may be grouped conveniently under three headings: (1) Permanent hermaphroditism: which covers those cases in which both ovary and testis develop and remain together throughout life.

(2) Gynandromorphism : cases which come under the previous heading but which exhibit a unilateral arrangement of male and female accessory and secondary sexual organs as well as of the ovarian and testicular tissue. It is convenient to consider these separately in Chapter XIII for theoretical reasons, although it is not strictly logical. (3) Sex-reversal : animals in process of transforming from one sex into the other necessarily pass through an hermaphrodite condition, but this is a transitory stage. The next two chapters will be devoted to these cases.

Normal Hermaphroditism.—The vertebrates provide only four known examples of normal hermaphroditism in the adult. Two of these are not functional hermaphrodites. One is self-fertilising. Three of these cases occur among fish and the fourth among the amphibia.

The first example is provided by the Elasmobranchs Heptanchus and Hexanchus. The females of these fish possess non-functional testes as well as ovaries. The condition in the female Heptanchus has been described by Daniel [64]. A rudimentary testis is present in *H. maculatus*, and is situated in the ovarian mesentery (mesovarium) at the anterior end of the ovary, to which it is parallel. This rudimentary testis consists of a larger anterior portion and a smaller posterior part which extends almost the entire length of the ovary. This hermaphrodite condition of the female is similar to that described by Semper in Hexanchus.

Two other interesting examples are provided by the Teleosts. Members of the genus Serranus have been known since ancient times to be hermaphrodite. Aristotle (*Aristotelis opera omnia, De animalibus historiæ*, lib. VI, cap. 13, edition Firmin Didot, 1854) says that the χαννη (*Serranus cabrilla* and possibly *S. scriba*) can conceive of themselves, and that among the individuals of the species it is doubtful that there are males and females, because all those caught have eggs. Pliny and Ovid also refer to it. These conclusions are confirmed and extended by Dufosse [68] and Brock [43]. The ovary consists of two hollow sacs united posteriorly where they are joined with a common oviduct. The testis is

considerably smaller and consists of an annular band of tissue embedded in the wall of the ovary at the posterior end. The spermatozoa and ova are matured and liberated at the same time. There can be little doubt that self-fertilisation usually occurs and is effected in the sea-water immediately after laying. These details appear to be true of three species : *Serranus cabrilla*, *S. scriba*, and *S. hepatus*.

Brock also describes *Chrysophrys aurata*, an Australian Teleost, which is hermaphrodite. The testis in it is as large as the ovary. The ovarian portion of the gonad is anterior and is partly surrounded by the testis. This species, unlike Serranus, does not appear to be self-fertilising, and the spermatozoa and ova appear to be matured at different times. Through the courtesy of Prof. J. P. Hill, F.R.S., I have been

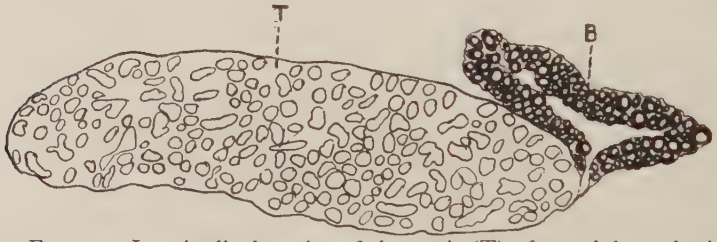
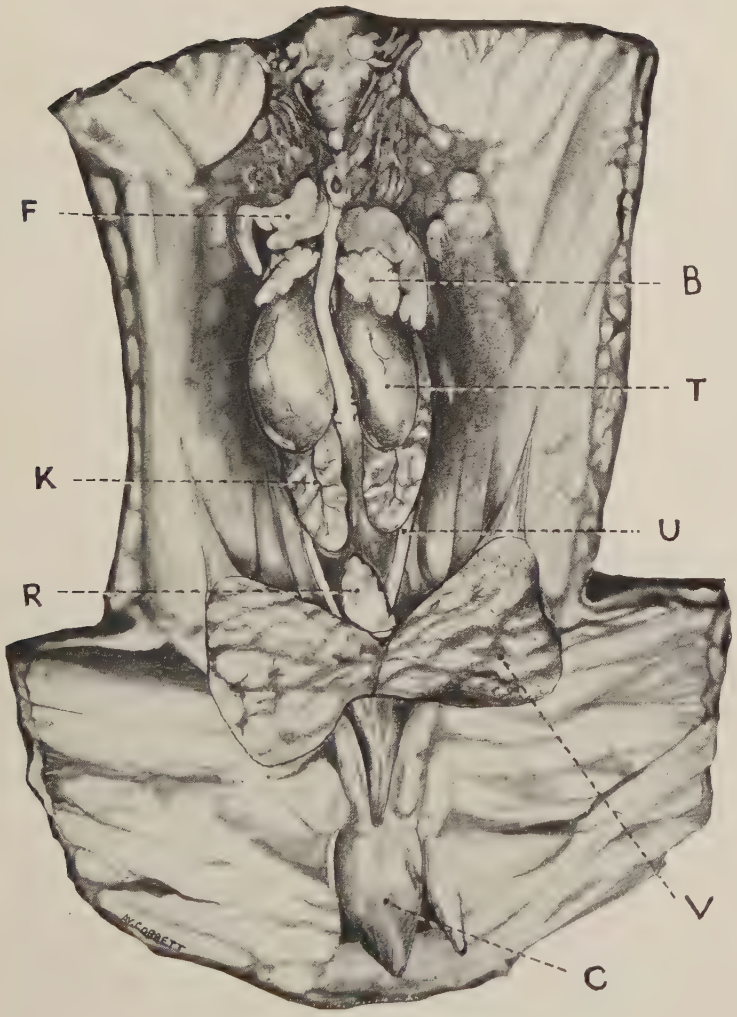


FIG. 15.—Longitudinal section of the testis (T) of an adult toad with Bidder's organ (B) attached to the anterior end. × 28.

enabled to examine sections of the gonad of a specimen in his possession which supports these conclusions. It is, however, highly desirable that the morphology, and especially the development, of both Serranus and Chrysophrys should be reinvestigated. Information regarding the early differentiation of the ovarian and spermatic tissue would be extremely interesting and might yield results of considerable importance.

The toads furnish another example of normal hermaphroditism, in this case of the adult male. They possess a rudimentary ovary, known as Bidder's organ, situated at the anterior end of each gonad to which it is usually closely attached (Fig. 15 and Pl.XVIII). Bidder's organ in the adult resembles in structure an immature ovary and contains many small oocytes which sometimes develop traces of yolk. It often equals the adult testis in



DISSECTION OF A MALE TOAD

The testes (T), Bidder's organs (B), and fat bodies (F) are shown. C, cloaca ; K, kidney ; R, rectum ; U, ureter ; V, bladder. × 3.

size, but is very variable. It is easily distinguished from the ovary (Pl. XIX) by its compact, scarcely lobed structure, its small size, and its golden-yellow to red colour. The Bidder's oocytes contain the yellow pigment which distinguishes them from the white ovarian oocytes of the same size. Moreover, large pigmented oocytes are never present. The accompanying table contains the available records of its occurrence in the Bufonidæ, to which it is confined.

TABLE XI.—OCCURRENCE OF BIDDER'S ORGAN IN THE BUFONIDÆ.

Species.	♂	♀
Bufo calamita, Laur.	+	+
Bufo vulgaris, Laur.=cinereus	+	+
Bufo viridis, Laur.=variabilis, Laur.	+	+
Bufo lentiginosus, Shaw. var. americanus and var. musicus	+	
Bufo crucifer, Wied.=ornatus	+	o ?
Bufo marinus, L.=maculiventris, =agua, =lazarus	+	o ?
Bufo intermedius, Gthr.	+	
Bufo compactilis, Wiegm.	+	
Bufo valliceps, Wiegm.	+	
Bufo regularis, Reuss	+	+
Bufo melanostictus	+	o ?
Bufo scaber		o ?
Bufo granulosus, Spix.		o ?
Bufo arenarum, Hensel		o ?
Bufo d'orbignii, Dum. and Bib.		o ?
Bufo kelaarti, Gthr.		o ?
Bufo taitanus, Ptrs.		o ?
Bufo quadriporcatus, Blg.		o ?
Bufo variegatus, Gthr.		o ?
Bufo typhonius	+	+
Bufo microtympanum	+	o ?
Bufo asper	+	o ?
Pseudophrynus	o	o
Nectophryne hoosi, Blg.	+	
N. afra, Bushh. and Ptrs.		o
Rhinophrynus	o	o

This table has been compiled from data published by Ponse [193] and by Subba Rau and Gatenby [196].

It can be seen from this table that Bidder's organ has been found in the males of all species of Bufo examined, and in one species of Nectophryne. It has been found in the females of five species of Bufo only, and in some of these it disappears

in the adult. Further examination of other species in which it has not been found in the adult female will probably reveal its presence in the young female. It is apparently absent in both sexes in the genera Pseudophrynus and Rhinophrynus, and is not found outside the family Bufonidæ.

Abnormal Hermaphroditism.—The occasional occurrence of hermaphrodite individuals has been described in all vertebrate classes. Only cases in which there is no reason to believe this condition to be transitory are included in this chapter. No attempt is made to treat the subject exhaustively, but the papers referred to provide further references.

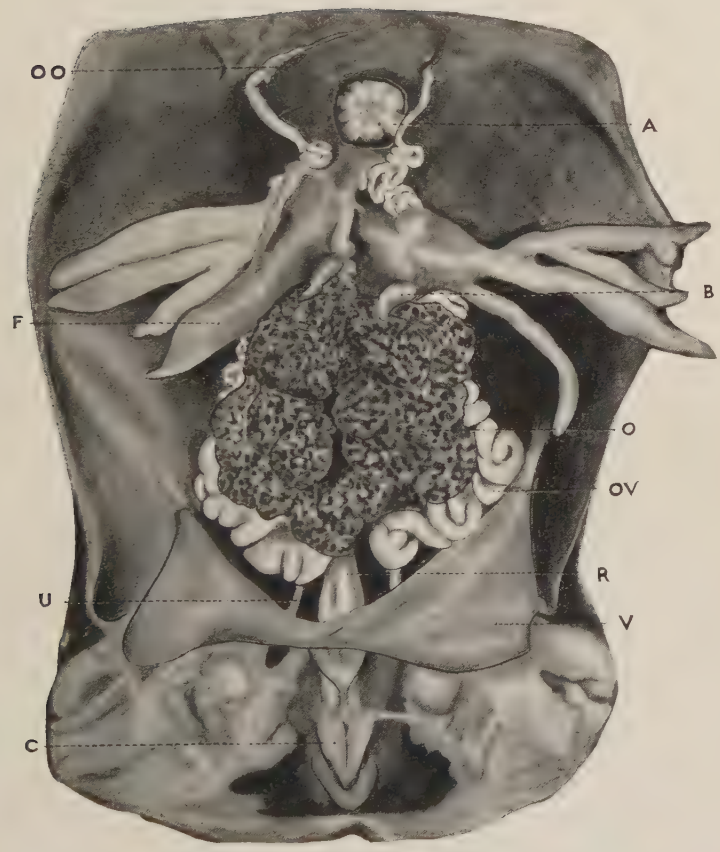
Cephalochordates.—Hermaphroditism is extremely rare in this class, only three cases being available. The first example is recorded by Langerhans [137] who detected tails of spermatozoa among the ova in the gonads of an Amphioxus. Two other cases are recorded, by Goodrich [95] and Orton [167] respectively, in which all the gonads except one were testes. This single ovary in both cases was on the left side.

Cyclostomes.—It appears probable, in the absence of evidence to the contrary, that hermaphrodite Cyclostomes are individuals in process of sex-reversal. Consequently further consideration of them will be deferred to the next chapter.

Pisces.—Abnormal hermaphroditism is extremely common among fish, and many examples are recorded. A number of examples are known in the codfish. Six are described by Weber and one by Howes [114]. Ovaries were present on both sides in all cases and testes in one. The testis was on the right side only in four and on the left only in two. The testis was attached to the ovary posteriorly in five of these and anteriorly in the other two.

An hermaphrodite trout is described by de Beer [12] with a normal testis on one side and an ovotestis on the other. The ovarian elements were limited to the cranial portion of the ovotestis.

Amphibia.—The normal but incipient hermaphroditism of the male toad has been described already, but further complications occasionally occur as abnormalities. Ponse [193] described seven toads with an ovary and a testis on one side



DISSECTION OF A FEMALE TOAD

The ovaries (O), Bidder's organs (B), and the large fat bodies (F) are shown. The oviducts (OV) with their ostia (OO) at the anterior end of the body cavity can be seen. A, œsophagus cut across. Other letters as in Plate XVIII. × 1·5.

as well as Bidder's organ. Two other cases are described by Cerruti [51] and Knappe [132] respectively. An analysis of these is given in the accompanying table. Bidder's organ, ovary, and testis are indicated by their initial letters and the formulæ are given in the order of succession of these organs antero-posteriorly.

Table XII.

No.	Authority.	Right Gonad.	Left Gonad.
1	Cerruti [51]	B+T+B+T	B+T+B+T
2	Ponse [193]	B+T	B+T+B+T
3	Knappe [132]	B+O+B+T	B+O+B+T
4	Ponse [193]	B+O+B+T	B+O+B+T
5	,,	B+O+T	B+O+T
6	,,	B+O+T	B+O+T
7	,,	B+O+T	B+T
8	,,	B+O+T	B+O
9	,,	B+O+T	B+O
10	Cerruti [51]	B+O+T	B+O

It is obvious that in every case the order of succession from the anterior end is Bidder's organ, ovary, testis, when all three are present. The testis invariably appears to be developed posterior to the ovarian tissue, whether this is true ovary or Bidder's organ. This fact appears significant in relation to the problem of embryonic differentiation and will be referred to at the end of the chapter. The first four cases exhibit a remarkable duplication of Bidder's organ on each side accompanied by an ovary and a testis or by two testes. Ponse [193] suggests that this condition might have arisen by an early separation into two parts of the germinal ridge. Each part would then develop separately and would form an ovary or a testis, the anterior zone of which would differentiate into an organ of Bidder. Alternatively it is suggested that the apparent intercalation of a testis between two organs of Bidder may not be real, but that the two portions are in reality a single organ of irregular shape. Cerruti [51], Spengel [211], and King [125] record cases in Bufo with an anterior ovary and a posterior testis on each side but without Bidder's organ. It

is probable that these have arisen by a transformation of Bidder's organ into a true ovary.

Many hermaphrodite frogs are recorded, but in the light of the recent results it appears probable that all these may be in process of sex-reversal.

Reptilia.—Hermaphroditism appears to be extremely rare among reptiles, and very few cases are recorded. A good example in the tortoise has recently been described by Matthey [155]. The animal was a male *Emys europæa*. There was a normal testis and epididymis on the right, and an ovotestis on the left side. A large oocyte formed the anterior end of this ovotestis, which also contained another large oocyte and some small ones. This gonad exhibited intense spermatogenesis, but no ovogenesis. The left epididymis was normal. The epididymis on each side was filled with ripe spermatozoa. Two oviducts similar to those of a young female were present.

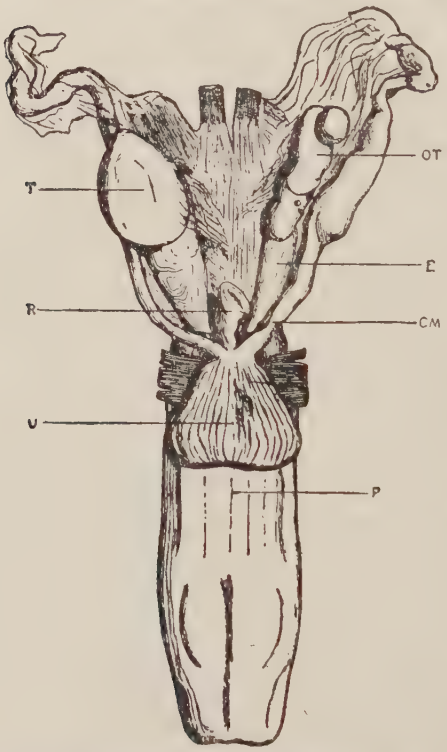


FIG. 16.—Reproductive organs of an hermaphrodite tortoise. CM, Müllerian duct; E, epididymis; OT, ovotestis, P, penis; R, rectum; T, testis. U, bladder. × 1·6. (From Matthey [155].)

Aves.—Hermaphrodite birds have been known since ancient times, and many examples are described. It appears that the majority of these are females in the course of transformation into

males, and therefore come within the scope of Chapter XII. One or two cases, however, appear to be permanent hermaphrodites (see also Chapter XIII). An example is described by Hartman and Hamilton [107]. This bird was a Rhode Island Red. It appeared to be a normal pullet for the first year and then the comb and wattles began to grow and the bird learned to crow. It laid once whilst being stroked by the owner. Its behaviour was in some respects male, in others female. It was killed when nine years old. At that time the head furnishings were male in character and there was a well-developed spur on the right leg. The plumage, however, was completely henny. An ovotestis was found on dissection on the left side and a testis on the right. A coiled oviduct and a thin, straight vas deferens were present on the left and a similar vas deferens (but no oviduct) on the right. The ovotestis contained normal oocytes up to 20 mm. in diameter. Ripe spermatozoa were present in the spermatic tubules of both the testis and ovotestis. This bird is of unique interest in that it possessed functional ovarian and testicular tissue at the same time.

Mammalia.—A large number of examples of true hermaphroditism in mammals are known, although its occurrence in this group must be considered relatively very rare. It appears to be much more frequent in certain forms, such as the pig, goat, and man, than in others. No case in the mouse or rat is known, although these animals are used in large numbers in laboratories, where its occurrence would probably be detected.

The pig furnishes by far the most definite and clear-cut examples. Five cases are described by Pick [191] and four others by Baker [8, 9], who gives also a tabulated list of recorded cases from which the appended summary (p. 158) has been made. It can be seen that almost all possible combinations of ovaries, testes, and ovotestes have been observed, although, in the majority of cases, the right gonad is more male than the left.

Hermaphrodite pigs appear to occur more commonly in certain strains than in others.

The typical arrangement of the gonads and ducts is shown

TABLE XIII.

Right Side.	Left Side.	No. of Examples.
Testis.	Ovary.	5
Ovary.	Testis.	1
Ovotestis.	Ovary.	3
Ovotestis.	Testis.	1
Ovotestis.	Ovotestis.	7
Ovary and Testis.	Ovary and Testis.	1

diagrammatically in the accompanying figure (17). The structure of the ovotestes is remarkably constant. The ovarian tissue always occurs in the same place (see Fig. 17) in relation to the testicular part, on its ventro-antero-median surface. Although

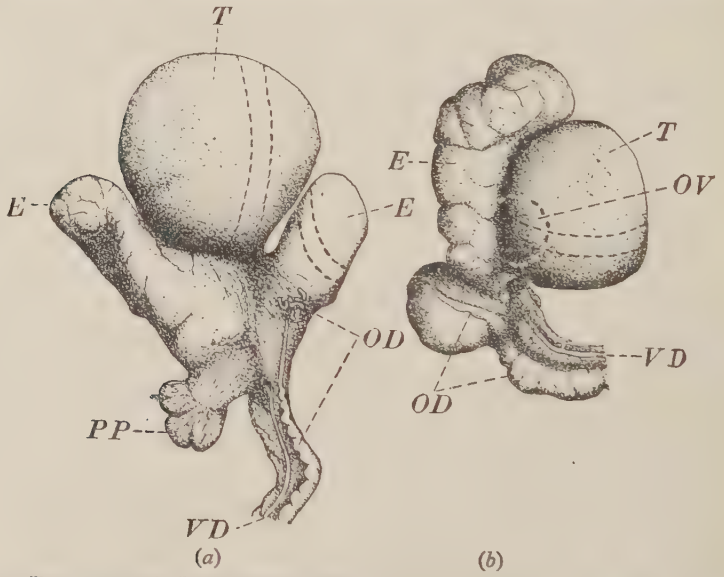


FIG. 17.—(*a*) Left gonad (testis) of hermaphrodite pig. (*b*) Right gonad (ovotestis). T, testis; Ov, ovary; E, epididymis; OD, oviduct; PP, pampiniform plexus; VD, vas deferens. The double dotted line across each gonad indicates the place of section. × 1·25. (From [31].)

the relative size of the two portions varies considerably the testis is usually the larger. It is significant that the ovary forms a cap on the surface of the testis, and that the tunica albuginea of the latter is continuous beneath the ovary. The

ovary is therefore outside this well-developed tunica which divides it from the testis. Histological study of a typical

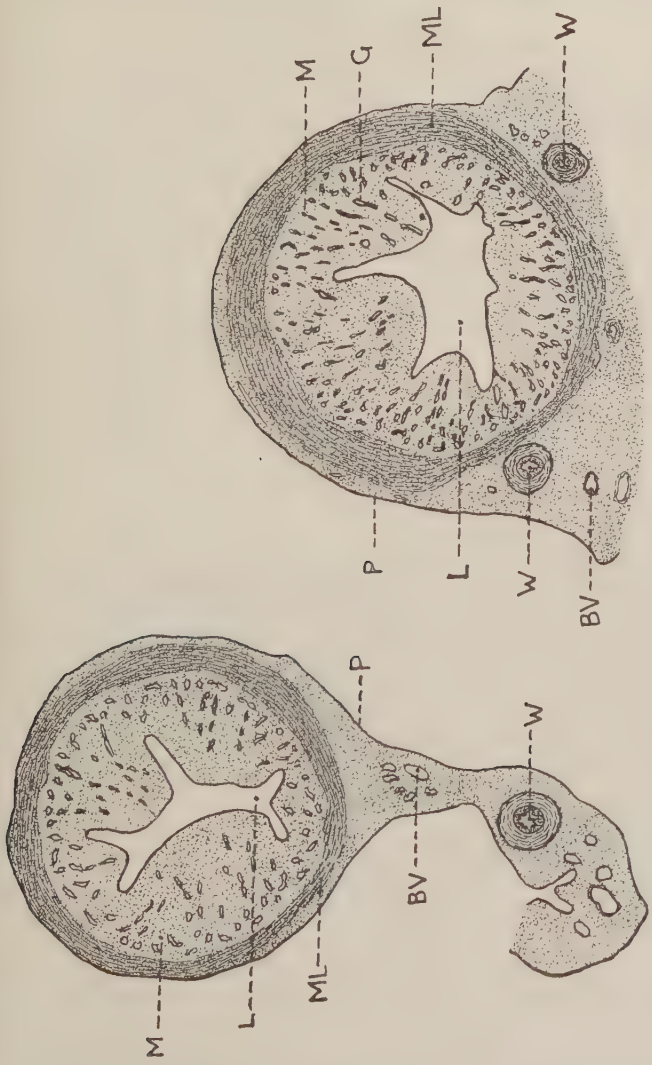


FIG. 18.—Transverse sections of the cornu (*a*) and the median portion (*b*) of the uterus of an hermaphrodite pig. BV, blood vessel; G, uterine glands; L, lumen of uterus; M, mucosa; ML, muscle-layers; P, peritoneum; W, vas deferens in broad ligament. × 14.

hermaphrodite pig has furnished interesting results of theoretical importance [31]. The right gonad was an ovotestis and

the left was a testis. Both gonads consisted of a medulla of spermatic tissue (Fig. 19) surrounded by a well-developed

FIG. 19.—Part of a lobule from the medulla of the ovotestis of a pig, showing the spermatic tubules (ST) and interstitial cells (IC). × 380.

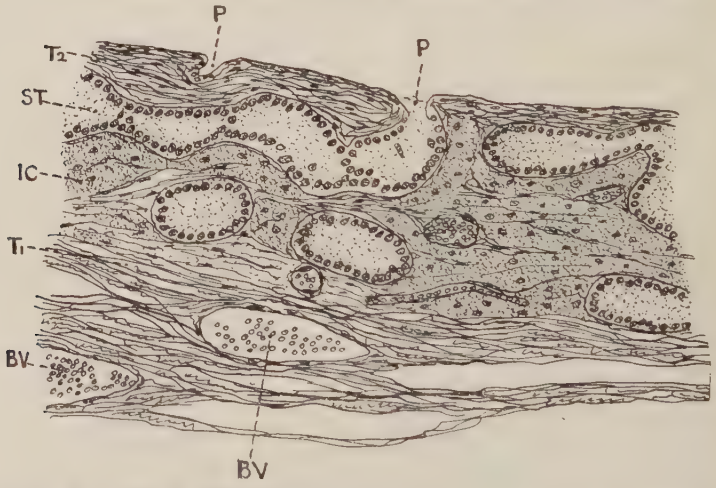


FIG. 20.—Part of the spermatic portion of the cortex of the ovotestis of a pig, showing the spermatic tubules (ST) opening by pores (P) on the surface. BV, blood vessels; IC, interstitial cells; T_1, primary tunica albuginea; T_2, secondary tunica albuginea. × 180.

tunica albuginea and, outside this, a cortical zone of spermatic tubules. The ovarian tissue composed a small part of the

cortical zone of the right gonad. The spermatic tubules of both cortex and medulla were sterile. Those of the cortex opened by distinct pores on the surface of the gonads (Fig. 20). Interstitial tissue was plentiful. The ovarian tissue was normal and contained numerous small oocytes in primordial follicles. The region of the cortex between the spermatic and ovarian regions was intermediate in character. Many of the tubules in this region contained true oocytes (Fig. 21). The ovarian tissue was

FIG. 21.—(*a*) Three oocytes in a spermatic tubule in the region between the ovarian and testicular cortex of the ovotestis of a pig. (*b* and *c*) Oocytes in enlarged follicles resembling tubules from the same region. (*d*) Oocyte in a normal primordial follicle. × 460. (From [31].)

covered on the outside by a cubical epithelium which was absent from the spermatic regions of the cortex, its place being taken by a thin secondary tunica albuginea. A unique example with a separate ovary and testis on each side has been described by Walentowicz [224].

Jaffe and Papanicolaou [121] described a guinea-pig with a

testis on the right and an ovary on the left side. An hermaphrodite cat with an ovotestis on the left is described by Harman [102]. The relations of the ovarian and testicular tissues in this cat were not well defined, as in the pig, a fact which is also true of the ovotestes described in the goat (Krediet [135]) and in man. The majority of cases of so-called hermaphroditism in man can be dismissed as examples either of intersexuality of the accessory organs or of arrested development. A few, however, appear to have had true ovotestes. A very clear example is that recorded by Salen and described by Pick [191]. This individual was forty-three years of age. The external genitalia were female in character, but the clitoris was large and penis-like. A vagina, uterus, and Fallopian tubes were present, but no vas deferens or epididymis. The gonad on the left side was an ovary with oocytes and Graafian follicles, while that on the right was an ovotestis. The ovarian portion of this ovotestis was apparently normal and contained follicles. The testicular portion, which was fairly well defined, contained spermatic tubules and a well-developed rete testis. The spermatic tubules were lined by a single or multi-layered epithelium, which contained germ-cells, and were with or without a lumen. Pick also gives a critical analysis of three other cases which appear to be genuine hermaphrodites. In each case the right gonad only was available and proved to be an ovotestis. The first was twenty years old. The ovarian portion of the gonad, which was found in an inguinal hernia, contained primary follicles in a typical cortical stroma. The testicular portion contained spermatic tubules, which showed no signs of spermatogenesis and were obviously degenerate. An epididymis and vas deferens as well as a Fallopian tube with an ostium, were present on the right side. There was no uterus or vagina and the male external genitals exhibited peni-scrotal hypospadia. The second case was forty years of age. The right gonad was removed from an inguinal hernia. The ovarian portion had a normal stroma, but follicles appeared to be absent. The testicular portion, which was much the larger, contained a rete testis and spermatic tubules with a Sertoli-celled epithelium but no germ-cells. A vas deferens

and epididymis were present on the right side. A vagina was present, but there were no uterus nor Fallopian tubes. The external genitals were of the female type and the clitoris was well developed. The third case was seven years old. The gonad was removed from an inguinal hernia. The ovarian portion contained primary and Graafian follicles. The testicular portion contained a poorly developed rete and spermatic tubules without lumina and containing Sertoli cells but no germ-cells. A vas deferens and epididymis, as well as a Fallopian tube with an ostium, were present on the right side. Uterus and vagina were absent, and the male external genitals exhibited peni-scrotal hypospadia. All these cases appear to be well authenticated. Three of them contained follicles, and all contained spermatic tubules in a degenerate condition similar to that commonly observed in undescended testes.

Theoretical Conclusions.—The occurrence of hermaphroditism affords proof that all vertebrates have the potentialities of both sexes, although, except in the rare cases of normal hermaphroditism, the organs of one sex only are developed. We have seen already that the sex-chromosomes probably afford the decisive factor in determining the development of the individual in the male or female direction. The occurrence of abnormal hermaphroditism in so many groups of normally diœcious forms indicates that this determination of sex may be upset in such a manner that ovary and testis can develop side by side. It is therefore necessary to assume that ovarian and testicular tissues are mutually compatible and do not inhibit each other. This assumption does not necessarily mean that the factors which determine the development of the indifferent germinal ridge into an ovary or into a testis are co-existent. They may be, on the contrary, quite antagonistic and never active simultaneously in the same individual. Indeed, they may be relatively positive and negative, one representing a real stimulus and the other its absence. Whether or not this is so, working simultaneously or consecutively they result occasionally in the production of ovary and testis in the same individual.

Abnormal hermaphroditism is much more frequent in some species of vertebrates than in others. For example, numerous hermaphrodite pigs are recorded, whereas no one has ever described a clear case of hermaphroditism in the mouse, although tens of thousands of mice are used in laboratories every year. It may therefore be assumed that while sex-determination is stable in some forms it is comparatively unstable in others. In other words, it is capable of quantitative expression although we are at present unable to assign definite values to it.

The examples cited in the present chapter are insufficient, with two exceptions, to warrant more detailed analysis. The toad and the pig, however, appear to provide a sufficient body of material for their examination. We will consider the former first.

The toad presents one definite feature in the constant presence of Bidder's organ in the male. This organ appears from its structure to be a true, though not functional, ovary. Moreover, as will be shown in the next chapter, it is capable of functioning as such under special conditions. The problem then presents itself as to how this ovarian tissue constantly develops in the male toad, and is always situated anterior to the testis. The embryological findings of Ponse [194], referred to already (Chapter VII), provide the necessary information. The germinal ridge in the toad gives rise to three primordia on each side: viz. pro-, meso-, and meta-gonads. These develop in an antero-posterior direction as in all other vertebrates. The progonad develops first, in the very young tadpoles, and subsequently atrophies completely. It contains pseudo-oocytes which never mature and forms a larval organ of Bidder. The mesogonad only starts development at metamorphosis and gives rise to the adult Bidder's organ. It exhibits active oogenesis at first, but later degenerates somewhat. The metagonad starts to develop at this stage, after metamorphosis, and forms in a female the definitive ovary, or in a male the definitive testis. It is obvious, therefore, that all individuals are under the influence of a female-determining stimulus up to the time of metamorphosis. This stimulus

remains operative in the chromosomal female and results in the differentiation of the metagonad into the definitive ovary. The male-determining stimulus in the chromosomal male only becomes operative at metamorphosis and determines the development of the still indifferent metagonad into the definitive testis. The mesogonad is not effected by the stimulus and retains its ovarian character. We may assume this to be due to its having passed the indifferent stage when it would be sensitive to the sex-differentiating stimulus. It can be seen that the facts can be accounted for by assigning a positive sign to either the male- or female-determining stimulus and a negative

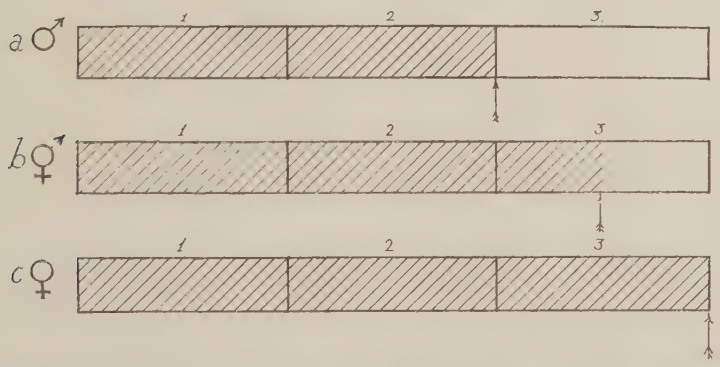


FIG. 22.—Diagrammatic representation of the effect of delay in the appearance of the male-determining stimulus in the toad. Sections 1, 2, and 3 represent the pro-, meso-, and meta-gonad respectively. The arrow indicates the point at which the male-determining stimulus begins to operate. The shaded portion indicates the part of the gonad which has differentiated into ovarian tissue before the appearance of the male-determining stimulus. The white portion indicates the part of the gonad which differentiates into testis, after the appearance of the male-determining stimulus and under its influence. (*a*) will be a normal male, (*b*) an hermaphrodite, and (*c*) a normal female.

sign to the other. They do not necessarily imply that both are of the same sign and therefore co-existent. This theory, on account of its simplicity, is exceedingly attractive and is capable of further application. The conditions of differentiation are shown diagrammatically in the accompanying figures. In Fig. 22 (*a*) the anterior part of the germinal ridge (progonad and mesogonad) have passed the indifferent stage and have

differentiated into ovarian tissue, as is always the case in the toad, before the male-determining stimulation is effected. Consequently only the posterior portion (metagonad) is able to develop into testis. This is the condition in the normal male toad. In Fig. 22 (*c*) the germinal ridge has differentiated into ovarian tissues before the male-determining stimulus is exerted, if it ever is. This represents the condition in the female. Should the male-determining stimulus become active prior to the differentiation of the whole gonad, but later than in the normal male, the condition would be represented by Fig. 22 (*b*). In this event the anterior portion of the metagonad would be ovarian and the posterior testicular. This would account for the abnormal hermaphrodite toads referred to in which an ovary is intercalated between the organ of Bidder and the testis.

This theory is applicable to other forms, as well as the toad, and may well account for hermaphrodites, such as the young Myxine, Serranus, Chrysophrys, etc., in which the ovary is anterior to the testis. It is obvious that for this arrangement to result the anterior extremity of the germinal ridge must be distinctly more precocious in development than the posterior. Moreover, it will be more frequent in forms in which sex differentiates very slowly, since a time factor is involved. These conditions are not realised in mammals where differentiation is rapid. In them the periphery of the gonad differentiates into ovary while the centre is still in the indifferent condition. The differentiation gradually extends inwards and backwards and results in the centre of the anterior region being in a similar stage of development to the periphery of the posterior region. Consequently a definite antero-posterior arrangement of ovary and testis could not arise as in the toad. The same theory is, however, applicable in principle.

The ovotestes of the pig conform closely to a single type. The ovarian portion forms a cap on the surface of the more or less spherical testis, and is always situated similarly relative to the other structures. It appears in the bend of the epididymis on the ventro-antero-median surface of the testis.

Unfortunately it is difficult to identify the part of the embryonic gonad from which this originated and to ascertain its true morphological relations. Another fact appears significant also. The testis portion is completely surrounded by the thick fibrous tunica albuginea. The cortical portion is outside this tunica, which separates it from the rest of the testis. It possesses a thin secondary tunica of its own beneath the germinal epithelium. The ovarian tissue forms part of this cortical zone, the remainder of which consists of seminiferous tubules which are still connected with the peripheral surface by distinct pores. The part of the cortical zone in the immediate vicinity of the truly ovarian portion, at least in one example,[31] contained spermatic tubules with oocytes in them. This region was, in fact, intermediate in character between the ovarian and testicular portions of the cortical zone. It is therefore apparent, from our knowledge of the organogenesis of these parts, that the medullary seminiferous tubules have been derived from the medullary cords, and the thick inner tunica albuginea surrounding them from the primitive tunica of the indifferent germinal ridge. Apparently the cortical zone was derived from the cortical cords which were formed outside the primitive tunica albuginea. Around this cortical zone the thin secondary tunica albuginea, corresponding to the definitive tunica of the normal ovary, formed. This interpretation is entirely in accord with our knowledge of development and with the structure of all the examples.

Differentiation of sex in mammals takes place after the formation of the medullary cords and of the primitive tunica albuginea. The testes differentiate first. The spermatic tubules develop from the medullary cords and the primitive tunica thickens and forms the definitive one. The germinal epithelium which has stopped proliferating does not recommence doing so, and finally degenerates. The ovaries remain in an indifferent stage for a short time after the testes have differentiated. During this period the females can only be distinguished by comparison with the males. Then the germinal epithelium again becomes active and proliferates the cortical cords. The growth of these disintegrates the primi-

tive tunica albuginea and forces the medullary cords to the centre of the gonad, where they slowly degenerate. The definitive ovarian tissue is formed on the outside from the cortical cords. It is therefore apparent that the male stimulus must be effected normally as soon as the medullary cords and the primitive tunica are formed, and before the cortical proliferation is due to commence. If this stimulus was somewhat delayed we might suppose that the cortical cords would have begun to form, outside the primitive tunica, before it was effective. This area of formation of cortical cords would centre around the anterior median ventral surface of the germinal ridge. Once formed their further development into ovarian or spermatic tissue would depend, probably, on the time of appearance of the male-determining stimulus. If this stimulus were effected at once the cortical cords would be transformed into a zone of spermatic tubules outside the primary tunica albuginea. Such cases would not be true hermaphrodites but would be intersexes, similar to those which have been described (Baker,[8, 9], etc.) with more or less abnormal genitalia but no ovarian tissue. If the stimulus was delayed still further a greater or lesser part of the cortex would differentiate into ovary. The appearance of the male stimulus then would result in a transformation of that part of the cortex, which had not completely differentiated into ovary, into spermatic tissue. The cortical cords of this portion might still be connected to the peritoneal epithelium and would possibly remain so. This would account for the peripheral connection and the open peritoneal pores actually observed in the cortical spermatic tubules of such an hermaphrodite. The cortical cords in the vicinity of the differentiated ovarian tissue would tend to be partly differentiated into ovary and would be transformed by the male stimulus into the zone of intermediate character with spermatic tubules containing oocytes.

The medullary cords, not having had time to degenerate extensively, would give rise to the spermatic tubules of the testis, and the primitive tunica would persist and thicken and would separate the testicular from the ovarian portion of the

gonad. An ovotestis, such as those found in the pig, would be formed on this hypothesis. It is interesting to note that a condition approaching this actually occurs normally in the male mouse embryo but is transient [28]. After the spermatic tubules have begun to form and the primitive tunica is thickening, the germinal epithelium continues to proliferate. A little mass of cells, undoubtedly corresponding to the cortical cords of the female, is formed in this way outside the tunica albuginea (Pl. XIII, Fig. 2). These always disappear in the mouse. If, owing to the male-determining stimulus being slightly delayed, these become rather more developed, it seems probable that an ovotestis of the pig type would be formed. Apparently sex is so stable in the mouse that this never occurs, but the explanation is suggestive in connection with the pig.

A similar condition has been observed by Humphrey [116] in male larvæ of Amblystoma. He found that an ovarian cortex was formed in many cases, outside the testis (Pl. XX, Fig. 4). This ovarian portion was usually limited to the ventral surface, but sometimes almost surrounded the testis. The ovarian tissue did not persist in these male embryos, but was a transitory structure like the incipient cortex in the mouse.

The fact that hermaphrodite and intersexual pigs seem to occur comparatively frequently in certain strains, whilst they are very rare in others, indicates that an hereditary factor is involved in their production and that it is probably quantitative. It will be seen that a similar assumption is necessary to account for the conditions in the frog.

The remarkable pig described by Walentowicz [224] is probably comparable to the other examples with an ovotestis on each side. It is easy to imagine that the forces which bring about the descent of the testis in a normal individual might result in the separation of the ovarian and testicular portions of the ovotestis of an hermaphrodite. The ovaries would then remain in their normal position whilst the testes would descend more or less completely, as was the case in Walentowicz's pig.

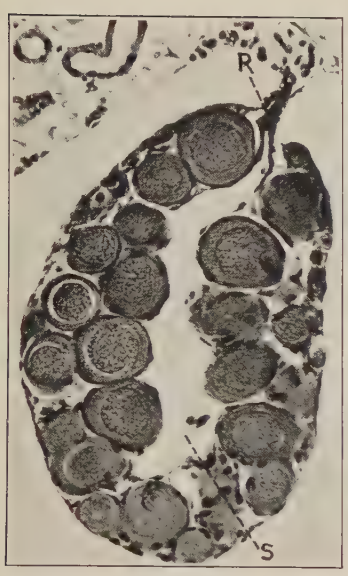
These hypotheses do not take into account the unilateral arrangement of the organs found in many hermaphrodites,

PLATE XX

Sex-reversal in Amphibia

1. Ovary of a normal 39 mm. frog (*R. sylvatica*) showing the rete (R) swollen in the ovary to form an ovarian sac (S). × 175. 2. Gonad of a frog about the same age which is in process of transformation from an ovary into a testis owing to having been reared at a high temperature. The degenerating ovarian cortex (O) and the nest of spermatogonia (SP) forming around the ovarian sac (S) are shown. × 265. 3. Testis of a normal male frog about the same age as Figs. 1 and 2. The absence of a central sac is obvious. The distinctly formed seminal tubules (ST) and the rete (R) can be seen. × 175. 4. Testis of larval Amblystoma showing the spermatic portion (T) partly surrounded by a thick cortical zone (C). The membrane separating these two portions has been picked out in black. The germ-cells of the spermatic portion are all spermatogonia, while those in the cortex have leptotene and pachytene nuclei. × 88. (Figs. 1, 2, and 3 from Witschi [247, 248]. Fig. 4 from Humphrey [116].)

PLATE XX



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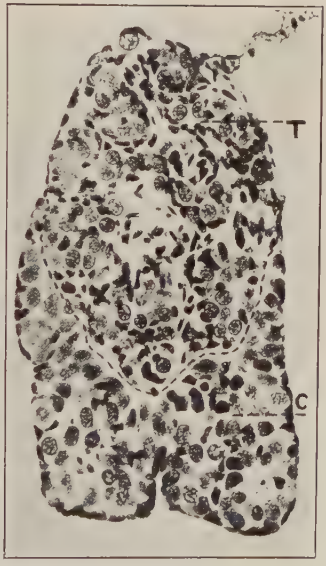


4

where an ovary is present on one side and a testis on the other, or an ovotestis on one side and an ovary or a testis on the other. It is interesting that, in the majority, the right gonad is more male than the left. Many authors have attempted to show that one side or the other has, in this respect, a preponderant tendency towards one sex, but the number of examples available are insufficient to provide a significant result. It is desirable that more data, relevant to this problem, should be collected. Since the gonads of the two sides develop approximately simultaneously in the vertebrate embryos, it is difficult to attribute this form of hermaphroditism to a single change over, during development, of the sex-determining stimuli. It must be admitted that this problem is at present unsolved.

CHAPTER XI

SEX-REVERSAL IN FISH AND AMPHIBIA

The sex-chromosome theory postulates that sex is determined at fertilisation. This determination is normally absolute and irrevocable. Until recently it was thought to be always so, although it was realised that in hermaphrodites the chromosomal mechanism must have been upset in some way.

During recent years an increasingly large body of evidence has been accumulated which shows that sex may be reversible and that an individual with the chromosomal constitution of one sex can develop the organs of the other sex. This reversal of sex may take place early in development before the sexual organs differentiate or it may take place at any subsequent time. Many forms exhibit an hermaphrodite condition during development and later change over in one or the other direction and become perfect males or females. Such cases are generally termed *juvenile hermaphrodites*.

Sex-reversal has been claimed to afford evidence against the sex-chromosome theory by its opponents. It is, however, a curious anomaly that it has afforded some of the strongest corroborative evidence in favour of the chromosomal determination of sex, as will be seen from the breeding results.

Cyclostomes.—Cunningham [60] found that males in *Myxine glutinosa* were extremely rare. Out of hundreds of specimens examined he succeeded in identifying only eight males. He found, however, that in all the females with very immature eggs the caudal portion of the gonad was testis. This testis even showed spermatogenesis and spermatozoa in one case. The females with well-developed ovarian oocytes had, on the other hand, no testicular portion, with a single exception. He concluded that the females were protandrous

hermaphrodites and that normally they effected fertilisation as males while young. Subsequently the testis disappeared as the ovary matured. Nansen [162] thought that all the young were males up to a length of 32–33 cms. and that they changed subsequently into females.

The careful investigations of Schreiner [205] show fairly conclusively that the earlier workers were mistaken and that the sexes in Myxine are distinct. Both sexes pass through an hermaphrodite stage in development. The testes in the males develop from the posterior portions of the gonads. The anterior portion of the gonad either fails to develop or contains oocytes arrested in development and showing signs of degeneration. The testes usually contained a few oocytes and sometimes many. The ovaries in the females developed from the anterior portions of the gonads, while the posterior or testicular portions were arrested in development and were sterile. These findings have been confirmed by Cole [53]. Conel [54] found a similar condition in Bdellostoma.

Okkelberg [166] has shown that the brook lamprey (*Petromyzon planeri*) passes through a similar hermaphrodite stage. This condition is found in all individuals and extends from about the 35-mm. long to the 70-mm. long stage. The oocytes and spermatic pockets are not segregated to the anterior and posterior ends respectively, but are both developed throughout the length of the gonads. The future sex can only be diagnosed by the relative frequency of the oocytes and spermatic cysts. Some individuals appear to fluctuate first in one direction and then in the other before the sex is finally established.

This unstable condition of sex in the young Cyclostomes is very interesting and is comparable to that found in the frog. Unfortunately sex-chromosomes have not been demonstrated in Cyclostomes up to the present time. The definite segregation of the ovarian elements in the cephalic end and of the testicular tissues in the caudal end of the gonad in the young Myxine suggests a similar developmental mechanism to that of the toad.

Pisces.—Huxley [117] has drawn attention to the fact that abnormal sex-ratios can be explained in some cases by the

assumption of sex-reversal of some of the individuals of a previous generation. He described a case in the Millions fish (*Girardinus*) which produces a brood of young every fourteen days. The sex ratio for about nine to ten months was 3 ♀ : 1 ♂. Then this ratio changed over for a few weeks to 1 ♀ : 1·5 or 2 ♂. Finally the ratio returned to the normal 1 ♀ : 1 ♂. Huxley pointed out that sex-reversed individuals mated with normal individuals in normal conditions will always give a preponderance of the sex other than that to which they belong. If the factor producing sex-reversal is operative over several generations and then disappears the result will be a decreasing preponderance of the one sex during this period, then a preponderance of the opposite sex in the first generation after the close of this period, and finally the normal sex ratio in succeeding generations.

These expectations can be shown easily in mathematical form. They explain the results in the Millions fish on the assumption that the XX♀, XY♂ formula is applicable and that 50 per cent. of the XY individuals were transformed into females. This would give the initial 3 ♀ : 1 ♂ ratio and the swingback would give 5 ♀ to 6 ♂. The actual ratios observed conform remarkably closely to these expected ratios.

Four cases of complete sex reversal in an adult Teleost, *Xiphophorus helleri*, have been described by Essenberg [73]. The male in this species is easily distinguishable from the female, since it possesses a gonopod or enlarged anal fin, which functions as an intromittent organ, and a sword formed of the modified ventral lobe of the tail fin. Sex-reversal of the female into the male is therefore clearly shown by the development of these secondary sexual characters in a known female. One of the four examples transformed without having produced any broods as a female. Another was the mother of three broods before reversal, but failed to produce any young as a male. The remaining two produced young before and after reversal. One of these produced a single brood before and, mated with a virgin female, five young after reversal. The sex-ratio of the latter was 2 ♀ : 3 ♂. The other produced two broods of fifty-three and forty respectively as a

female. It then ceased to breed and after about nine months had become a perfect male. It was mated with a virgin female and produced eight young, five of which died. The remaining three were 1 ♂ : 2 ♀. Unfortunately Essenberg merely states that the sex-ratio of the offspring of these fish before reversal was typical of the species and does not give exact figures. The ratios after reversal indicate that the female is heterogametic, but although the numbers are too small to give significant results they are in accordance with the expected ratio of 1 ♂ : 2 ♀. During reversal the ovarian tissues appear to degenerate completely and a new growth of sex-cords is formed from the peritoneal epithelium lining the ovarian cavity. These branch, acquire a lumen, and become the spermatic tubules. The testis of the transformed fish is identical in position and structure with that of a normal male. The oviduct becomes the sperm-duct. Harms has recorded,[105] also in Xiphophorus, the transformation of adult females into functional males.

These two examples of sex-reversal in fish are of particular interest since they appear to show that the female is the homogametic sex in Girardinus and the male homogametic in Xiphophorus. The small amount of cytological evidence available concerning the sex-chromosomes in fish appeared to indicate also (Chapter IV) that the heterogametic sex was the female in some forms and the male in others.

Mrsic[161] obtained several young Rainbow trout with ovotestes in cultures in which the eggs were over-ripe when fertilised. The ovarian portion of each of these ovotestes was anterior and the testis posterior. He believed that these fish were undergoing sex-reversal and that the gonad, at first an ovary, was transforming into a testis in a caudo-cranial direction. Huxley[118] obtained similar, though not such marked results. He calculated the probable errors for his own and Mrsic's figures, which are summarised in Table XIV. This evidence for this transformation is, however, hardly conclusive since the change in the sex-ratio on which it is based is barely significant. The anterior position of the ovary relative to the testis admits of a similar developmental

Table XIV.

Author.	Treatment.	Total.	♂ per cent.	Probable error.
Huxley	Control	119	47·90	±3·09
,,	Fertilised 4–7 days late	332	54·52	±1·83
Mrsic	Control	90	47·8	±3·55
,,	Fertilised 4–7 days late . . .	199	39·7	±2·34
,,	,, 13 ,, ,, . . .	77	50·6	±3·84
,,	,, 21 ,, ,, . . .	57	66·7	±4·21

interpretation to that applied in the last chapter to toads. This interpretation is applicable irrespective of whether the hermaphrodite condition is permanent, as in the toad, or transitory, owing to the degeneration of either the testicular or the ovarian portion, as in Myxine, or as Mrsic claims to be the case in his Rainbow trout.

Amphibia : Sex-reversal in Young Frogs.—Pflüger in 1882 discovered in cultures of certain local European races of frogs (*R. temporaria*) shortly after metamorphosis a remarkable preponderance of females. The sex-ratio in similar cultures of other races did not show an excess of either sex. He found that recently metamorphosed wild frogs from the same localities gave similar results. Adults from these localities did not show an excess of females. These results are summarised in Table XV.

Table XV.

Local race.	Percentage of males.			
	Six months old = recently metamorphosed.		Wild 1½ years old.	Wild adults.
	Culture.	Wild.		
Utrecht	12–14	13·2	—	48·8
Königsberg . . .	48·5	48·5	—	50
Bonn	35·7	36·3–35·5	49	49·4

Pflüger concluded from these results that in certain local races a considerable number of the young females normally

transform into males, since the change in the sex-ratio could scarcely be explained by a differential mortality.

Pflüger's discovery formed the basis for the brilliant researches of Richard Hertwig, and his pupils, Schmitt-Marcel, Kuschakewitsch, and Witschi, which have thrown much light on the problems of sex-determination in vertebrates.

Schmitt-Marcel, working on a local race from Dorfen (north-east of Munich), examined the sex-ratio at definite intervals after metamorphosis. His results, as summarised by Witschi [238], are given in the accompanying table.

TABLE XVI.—SEX-RATIO OF DORFEN RACE (SCHMITT-MARCEL, AFTER WITSCHI [238]).

Months after metamorphosis.	Percentage of females.	Percentage of hermaphrodites.	Percentage of males.	Number used.
0	85	—	15	225
1	85	—	15	310
2	75	8	17	390
3	70	12	18	332
4	70	12	18	215
6	64	18	18	80
10	55	24	21	220
12	54	24	22	210
13	54	20	26	200
14	54	15	31	200
15	53	12	35	180
16	53	7	40	200
18	52	4	44	120
22	52	—	48	200

These results place Pflüger's conclusion beyond dispute and show that in this race the transformations take place during the second to eighteenth month after metamorphosis. They also supply us with the material for estimating the average time taken for complete transformation. This very definite time limit is remarkable, and indicates that a time factor is involved in the transformation. The transformation may take place at a later stage or even in the adult in other races.

The frogs which are at first female and, becoming juvenile or Pflügerian hermaphrodites, transform into males are pre-

sumably genetically males. Since the male frog is heterogametic these juvenile hermaphrodites must be assumed to be heterogametic also. This indirect form of differentiation of the males appears to depend on the delayed appearance of the male-determining stimulus, which allows of the gonads becoming differentiated into ovaries first. The male-determining stimulus must be effective before the gonads have had time to differentiate into ovaries and while they are still in the indifferent stage in male frogs which exhibit the normal or direct form of development already described (Chapter VII). It is obvious, therefore, that a time factor is involved in the production of Pflügerian sex-reversal. Since the direct and indirect forms of male differentiation are racial characters, this factor must be hereditary. Further, since the proportion of males exhibiting the direct and indirect forms of development—in other words, the proportions of normal differentiated males and of Pflügerian hermaphrodites—is constant in any given race, and varies in different races, this hereditary time factor must be strictly quantitative. These conclusions are identical with the more important assumptions contained in Goldschmidt's hypothesis of sex-determination in racial crosses of the Moth, *Lymantria*. They are borne out in a remarkable manner by the results of the series of racial crosses made by Hertwig (see Witschi). These crosses also showed that both parents participated in the transmission of the sex-determining factors, since different results were obtained by crossing a single female with different males and *vice versâ*. It is not necessary to go into the details, which are elaborate, of these crosses. Witschi,[241-243] using Goldschmidt's hypothesis, was able to attribute relative values to the hereditary factors in the different races and to assign arbitrary numerical values to them. Applying these formulæ to Hertwig's results, he was able to show that the expected ratio of males, early differentiated males and females transforming into males, resulting from a given cross, approximated to the results obtained. The actual formulæ, which are rather complicated, can be referred to in Witschi's paper[241]. Space does not permit of their repetition here.

The process of indirect development of the male frog has been described in detail by Witschi [239, 247]. The gonads in the males, which undergo this type of development, differentiate into ovaries and subsequently transform into testes (Pl. XX, Fig. 2, and Pl. XXI, Figs. 1 and 2). This transformation may take place at any time, even in the adult. The method of transformation consists in the migration of germ-cells from the base of the germinal epithelium into the walls of the ovarian sacs, where they form nests, which differentiate into spermatic tubules. At the same time the cortical or ovarian portion of the gonad degenerates, leaving only a thin peritoneal epithelial covering to the gonad. The ovarian sacs then transform into the rete and vasa efferentia of the testis. The stage of transformation attained by the two gonads, or even in different parts of the same gonad, is frequently not the same. Moreover, the basal and central regions become testis before the peripheral ovarian region has degenerated. Ultimately the whole gonad is transformed into a testis.

Sex-reversal in Adult Frogs.—Adult hermaphrodite frogs are comparatively common and many examples are recorded. Crew [55, 56] has reviewed a number of these, together with five cases which he has himself described. He came to the conclusion that they were females in process of transformation into males. He based this conclusion on the signs of ovarian degeneration always present, and on the presence of extensive areas of ovarian pigment and the irregular contour of the testes which indicated their ovarian origin. Moreover, he was able to arrange the cases examined in a nearly complete series showing the successive stages in the process of transformation.

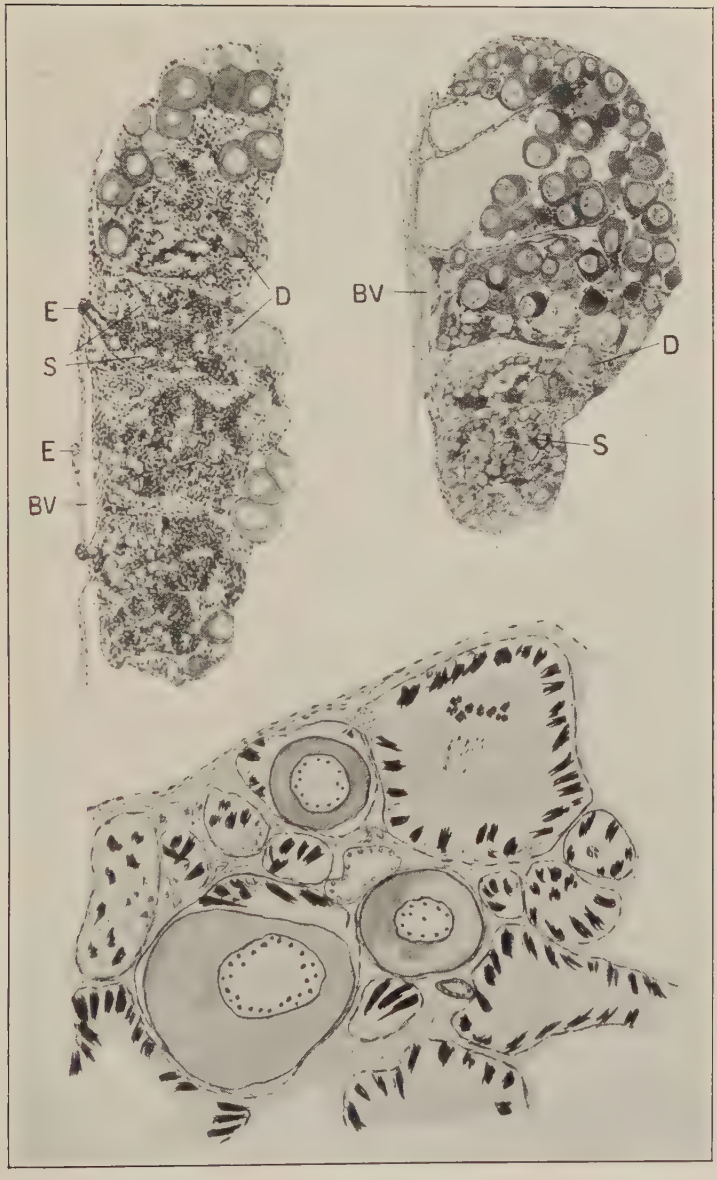
The hermaphrodite gonads of these adult frogs in process of transformation are completely different from those of toads. They invariably exhibit marked signs of ovarian degeneration and of active testicular development. The two tissues do not appear to be mutually compatible and able to co-exist in a stable condition in the one gonad, as they do in the hermaphrodite toad. It may be that the testicular development takes

PLATE XXI

Sex-reversal in Amphibia

1. (Top right) Longitudinal section of the gonad of a young frog transforming from an ovary into a testis. The anterior end is at the top and is normal for an ovary. The large ovarian sacs are characteristic. The posterior end is normal testis. The region between is in process of transformation and shows the formation of spermatic nests, the degeneration of the ovarian tissue and the collapse of the ovarian sacs. 2. (Top left) A similar gonad rather more transformed than Fig. 1. × 77. 3. (Below) Part of the testis of an adult male frog showing oocytes in spermatic tubules which also contain tufts of mature spermatozoa. × 85. D, degenerating ovarian elements ; E, vasa efferentia ; BV, blood vessel ; S, spermatogonia. (Figs. 1 and 2 from Witschi [245] and [237].)

PLATE XXI



place in consequence of the ovarian degeneration or *vice versâ ;* or both may result directly from a common cause. Another difference is to be found in the relative positions of the ovarian and testicular components, which are anterior and posterior respectively in the toad. The testis portion of the gonad of an hermaphrodite frog is almost always median to the ovarian portion. It appears that the first development of the spermatic tissue always takes place in the region of the hilum and spreads from there. The transformation may take place in both gonads simultaneously, but it begins more often on one side and later spreads to the other. When the latter is the case the transformation may begin in either the right or the left gonad with apparently equal probability.

During the transformation the Müllerian ducts atrophy to a greater or less extent, while the rete testis, seminal vesicles, and thumb pads develop. Ultimately the transformation is complete and the animal is a functional male only distinguishable from the normal by the irregular contours of the testes. Two good examples in the author's possession of frogs in process of transformation are shown in the accompanying Fig. 23. The right gonad of one (*b*) is an ovotestis, in which the ovarian tissue is divided into two portions, respectively, lateral and postero-lateral to the testis. The left gonad, which is the larger, is a testis. Both gonads are of irregular contour, unlike the ovoid testes of a normal male, and are divided into lobes by deep constrictions. The rete are well developed on both sides and are clearly shown on the left (*i.e.* right of figure). The oviducts are atrophied, but that on the right is the larger and is slightly convoluted posteriorly. Thumb pads were well developed. A section of the ovotestis showed spermatogenesis in active progress in the spermatic tubules, and spermatozoa were present. The ovarian part is obviously degenerating, as are the oocytes scattered sparsely in and between the spermatic tubules. The left gonad of the other (*a*) is an ovary and the right gonad an ovotestis with only a small nodule of spermatic tissue.

Results of Breeding with Sex-reversing Adult Frogs.—

The problem of the genetical sex-constitution of these adult sex-reversals is of interest.

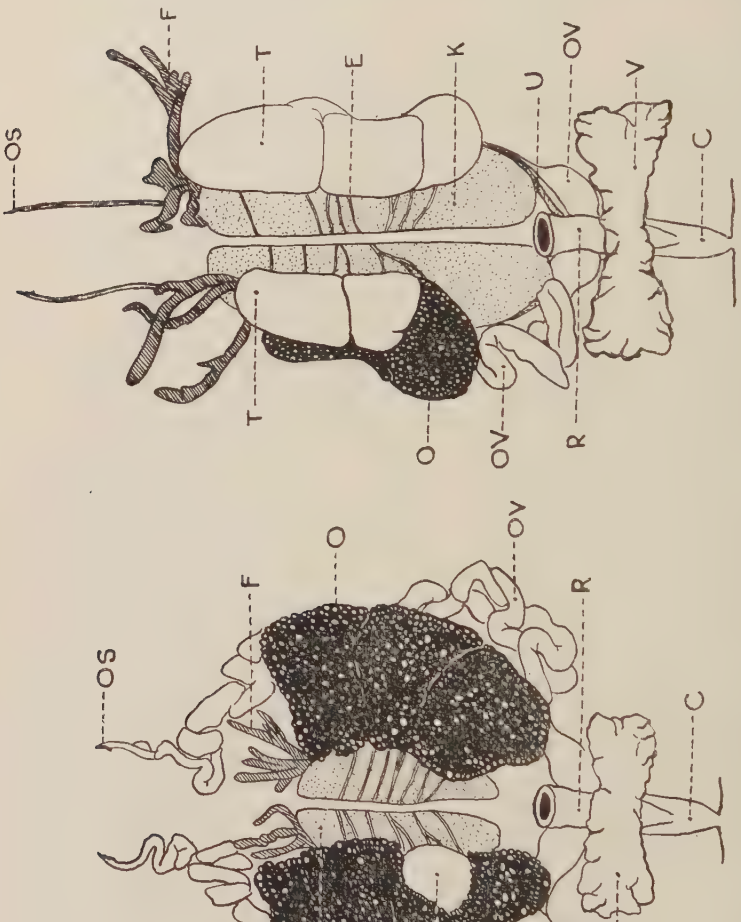


FIG. 23.—Adult hermaphrodite frogs. (*a*) Showing only a small nodule of testis (T) in the right ovary. × 1·7. (*b*) Showing a ridge of ovarian tissue (O) attached to the right testis. × 2·3. C, cloaca; E, vasa efferentia; F, fat body; K, kidney; OS, ostium of oviduct; OV, oviduct; R, rectum; U, ureter; V, bladder

It might be thought that they are chromosomal males, as

has been assumed in the case of the juvenile sex-reversals. The complete and functional nature of the transformation in the adults has rendered it possible to test this hypothesis directly by breeding experiments. These tests have shown that the transforming adults are chromosomal females and have confirmed the cytological conclusion that the female is the homogametic sex in frogs. Crew [56] obtained 774 young frogs, in which the sex could be determined, from an hermaphrodite mated with a normal female. All these young were females. Witschi [242] obtained two frogs which proved on dissection to be hermaphrodites. He fertilised the eggs of a normal female of the Davos local race, which exhibits early direct differentiation of the males, with sperms from the excised testis of the first of these. The young resulting consisted of 238 females and one male. The second hermaphrodite had ripe eggs in the uteri as well as spermatozoa in the testis. Both eggs and sperms were fertile and enabled him to make three crosses by the method of artificial fertilisation. He fertilised the eggs of a normal Davos female, and some of those of the hermaphrodite with sperms from the hermaphrodite. The remainder of the eggs of the hermaphrodite were fertilised by sperms from a normal Davos male. Finally he crossed the Davos male and female.

The results of these crosses are shown in Table XVII.

Table XVII.

Parents.		Offspring.		
♂	♀	♂	♂	♀
Davos ♂.	Davos ♀.	127	1	128
Davos ♂.	Hermaphrodite 2.	135	0	132
Hermaphrodite 2.	Davos ♀.	0	0	182
Hermaphrodite 2.	Hermaphrodite 2.	0	1	45

These crosses prove beyond dispute that this hermaphrodite and the normal female were homogametic, and the normal male heterogametic. The single hermaphrodite offspring of the self-fertilisation experiment was a typical case of sex-

reversal. This F1 hermaphrodite, and also that resulting from the cross of the other hermaphrodite, may have resulted from a direct inheritance of the hermaphroditism from its parent or from the fact that the eggs were somewhat over-ripe when fertilised.

We therefore arrive at the remarkable conclusion that while juvenile hermaphrodites must be assumed to be genetically males (heterogametic), adult hermaphrodites are, on the other hand, quite definitely genetically female (homogametic).

Experimental Sex-reversal in Frogs.—Apart from the hereditary factors concerned in sex-determination in the frog, external factors also appear to play an important part. Hertwig [109] succeeded in modifying the sex-ratio in cultures by keeping them at high and low temperature. The results were more definite when a race, in which a considerable proportion of the males develop indirectly, was used. He cultured the eggs of the Irschenhauser race, which is of this type, at 10° and 20°C. Those cultured at 10° produced 35 ♂ and 438 ♀, as well as a very few hermaphrodites and individuals which were not sexed. Those cultured at 20° produced nothing but 241 females. It is therefore clear that the cold had a significant effect in producing males. Hertwig obtained a similar result with *Rana esculenta*. Cultures at 30° produced 344 ♂ : 319 ♀, while those at 15°–18° produced 260 ♂ : 85 ♀.

Witschi [248] has recently extended these experiments to *Rana sylvatica*, in which the males normally differentiate early and directly. The sexes differentiate usually during the fourth week. The temperature of the experimental cultures was raised to 32° ± 2° C. at the end of the fifth week. The animals were all killed at from 15 to 33 days later. There were no normal females, 53 females in various stages of transformation into males, and 62 normal males. These results are in striking contrast to the controls, which showed a sex-ratio of 100 ♀ : 96 ♂. These experiments show that the high temperature brought about sex-reversal of all the females. The histological changes taking place in these transforming ovaries are also described.

Delayed fertilisation of the eggs also tends to produce

males. Kuschakewitsch (see Witschi [238]) demonstrated this very clearly. He obtained approximately equal numbers of males and females from a normal culture, but only males from a culture in which the eggs were eighty-nine hours over-ripe when fertilised. This experiment was quite conclusive owing to the small mortality experienced. The results are given in the accompanying table.

Table XVIII.

Time of fertilisation.	Per cent mortality.	Number sexed.	♂	☿	♀	♂ per cent.	♀ per cent.
Normal	6	111	58	0	53	53	47
Eighty-nine hours late .	4	300	299	1 lateral	0	100	0

It is clear that over-ripeness of the eggs might result in the production of males in several ways. It might act (*a*) by its influence before fertilisation on the maturation divisions, resulting in the extrusion in the polar body of both X-chromosomes, or (*b*) by causing selective fertilisation by male-determining sperms only, or (*c*) after fertilisation by over-riding the hereditary sex-determining factors.

The first of these hypotheses (*a*) involves the further assumption that half the offspring, resulting from eggs fertilised by Y-bearing sperms, will contain no X-chromosome. Since the mortality is so small it would be necessary to assume that these individuals survived. Presumably they would be capable of producing only male offspring, since half their sperms would have a Y-chromosome and half no sex-chromosome at all, but this result has not been realised. Since extremes of temperature produce a similar result to that of over-ripeness, although operative only after fertilisation, there is reason to suppose that the last (*c*) is the true explanation.

Experimental Sex-reversal in the Toad.—The toad differs from the frog as regards sex-determination in two important respects. First, the juvenile hermaphrodite condition is stable and persistent in the toad, whereas it is unstable and transitory in the frog. Secondly, sex-reversal takes place

in nature with comparative frequency in the frog, whereas it is not known in the toad. However, male toads have been transformed into females by experimental means. Harms [103] castrated young male toads and found that after a considerable time Bidder's organ transformed into an ovary. Ponse [193] obtained a similar transformation of seven males. The transformation of the organ of Bidder into an ovary appears to take two or three years. It is accompanied by a gradual hypertrophy of the oviducts which become convoluted and resemble those of a female. The oocytes in the Bidder's organ grow and develop yolk and pigment. They are then indistinguishable from the ovarian oocytes of a normal female. Both Harms and Ponse succeeded in getting these sex-reversed males to lay. Ponse mated six, three years after they had been castrated. Only two of these laid. The eggs were rather abnormal, owing to the compression exerted by the somewhat small oviduct, but were fertilised. The majority died at an early stage of development, but some lived and metamorphosed. Nearly 370 young toads were obtained in this way and sexed during three seasons. They were all males. This surprising result, which Mlle. Ponse has courteously communicated to me before publication, appears to demonstrate that the male toad, unlike the male frog, is homogametic. Mlle. Ponse has pointed out to me that this interpretation could be questioned on either of two scores. First, that the male employed in the crosses had an abnormal genetical constitution. This objection was removed by mating the male parent with a normal female, which resulted in a normal sex-ratio. Secondly, that delayed fertilisation, resulting from detention of the eggs before laying in the abnormally small oviducts, or their abnormal developmental conditions in the atypic Bidderian ovary, might transform the genetically female offspring into males. Mlle. Ponse has, however, shown that Bidder's organ develops into an ovary in females that have been ovariotomised. During the time when this development is taking place the oviducts atrophy and then partly regain their normal size. They consequently resemble those of the transformed males. Laying was retarded in these females, even more than in the

males, and the eggs, when laid, were compressed in a similar manner to those of the transformed males. Nevertheless, both male and female young were reared from them. Consequently this objection also is removed.

It must therefore be concluded that in the toad the male is homogametic, although the reverse is true of the frog. How this difference could have arisen and why it should exist in two such closely related forms as the frog and the toad, as well as in certain fish (see p. 175), remains at present inexplicable. H. D. King [127] succeeded in affecting the sex-ratio in toads by reducing the water-content of the eggs, before fertilisation by treating them with hypertonic solutions of salt or sugar, and at fertilisation by keeping them out of water but in a moist atmosphere. She employed the eggs from two females A and B. The mortality was remarkably low, and in all cases the percentage of females was above that of the controls. This female preponderance was specially significant in the cultures fertilised out of water. The results are summarised in the accompanying table.

TABLE XIX.

Parent.	Treatment.	Per cent. mortality.	Number sexed.	♂ per cent.	♀ per cent.
♀ A	Control	8	322	52·5	47·5
	2 per cent. sugar sol. 10 mins.	7	279	47	53
	2 per cent. salt sol. 10 mins.	12	176	40	60
	Out of water	5	381	28	72
♀ B	Control	4·5	334	53·5	46·5
	2 per cent. sugar sol. 20 mins.	22·5	194	44·5	55·5
	Out of water	6·5	374	23	77

This alteration in the sex-ratio in Bufo is comparable to the results obtained in cultures of frogs (p. 184) exposed to temperature extremes.

Other Forms of Sex-reversal in Amphibia.—Many authors have described the occasional occurrence of oocytes in the testes

of apparently normal frogs (Pl. XXI, Fig. 3) and toads. The recent investigations of Guyénot and Ponse [99], Welti [226], and of du Bois and de Beaumont [20] have shown that a regular wave of oogenesis occurs in grafted and regenerated fragments of testes in Bufo, Rana, and Triton. The grafted or regenerating spermatic tissue exhibits a primary phase of degeneration in the seminal tubules. About the time when this degenerative phase is at its height, varying from five to twenty months after the operation, oogenesis starts. The oocytes are formed within the spermatic tubules, apparently from spermatogonia, and become surrounded by follicles. They may be single or in groups completely filling a tubule, but seldom exceed a size of 200 μ in diameter. They may, in rare cases, develop yolk and pigment. Gradually, as the spermatic tissue regenerates and spermatogenesis restarts, these oocytes degenerate and disappear from the tubules. This remarkable condition of transitory hermaphroditism must be looked upon as incomplete sex-reversal of the adult male, and indicates that even differentiated and functional spermatic tissue retains the power of forming female germ-cells.

Some light on the origin of the oocytes in the testes of Amphibia may be derived from the interesting observations of Agar [1] on the testes of Lepidosiren, referred to in Chapter III. He observed oocytes and cells intermediate in character between them and spermatocytes in the testes of this fish. Apparently spermatocytes in diakinesis were capable of undergoing a growth stage which, if sufficiently prolonged, resulted in their transformation into oocytes. It is probable that the oocytes which are found in Amphibian testes arise in a similar manner.

A remarkable case of sex-reversal in an adult male newt (*Triton alpestris*), recorded by Champy,[52] affords the logical conclusion of this incipient transformation of Amphibian testes into ovaries. The annual incidence of spermatogenesis in males was prevented by prolonged starvation. It was found that the testes in these starved males, killed in spring, were replaced by strips of fat containing undifferentiated germ-cells. Under the influence of intensive feeding two such

starved males assumed the external characters of the female. One was killed in January, but the testes were similar to the other starved individuals. The other was killed in April, having assumed by that time an entirely female appearance. The fat-bodies, occupying the former sites of the testes, were found to contain an ovary and an oviduct on each side. This example appears to be a case of complete transformation of a testis into an ovary, but, since the cases are so few, it is desirable that the experiment should be repeated.

It is remarkable that sex-reversal, which appears at first contrary to the sex-chromosome theory, has provided the most powerful corroborative evidence in favour of it. The breeding results obtained from crosses with sex-reversed individuals can be explained on no other basis. At the same time, the results described in this chapter include almost every conceivable form of sex-reversal. The functional ovary can transform into a functional testis. The adult testis can exhibit a transformation in the female direction. Both genetic males and females of the same species may develop ovaries which later transform into testes. Finally, the chromosomal mechanism of sex may be completely different in two closely related forms, the male being heterogametic in one and the female in the other.

CHAPTER XII

SEX-REVERSAL IN BIRDS

The work dealing with sex-reversal in fowl is confined to two limited spheres, the first dealing with changes produced in the sex-ratio and the second with the natural or experimental transformation of differentiated female fowl into males. The latter of these is by far the more important and comprises a large amount of work to which the greater part of this chapter is devoted. Before proceeding to deal with this we will consider briefly the altered sex-ratios which Whitman and Riddle claim to have produced.

Altered Sex-ratios in Pigeons.—It has often been claimed, but not satisfactorily proved, that of the two eggs which pigeons lay as a normal clutch the first tends to produce a male and the second a female. Consequently a normal sex-ratio of approximately 1 : 1 results. Whitman and Riddle [201] claim to have profoundly modified this sex-ratio in pigeons by two methods. They maintain that crosses between species, genera, or families result in an increase in the number of male offspring produced, depending on the width of the cross. Thus crosses between families usually produce an excess of males. They also claim that overwork affects the sex-ratio. This was brought about by consistent removal of the eggs from a pair of birds as soon as laid, which results in a greatly increased production during the season. Moreover, all the eggs can be given to other birds for incubation and the sex of the resulting offspring can then be determined. The eggs of such a series derived from a generic cross produce chiefly males at first, and later in the season chiefly females ; finally towards the close they become incapable of development. The time of appearance of females

and of eggs incapable of development is reached earlier in successive seasons as the birds grow older. The results published so far certainly support these conclusions, but a final decision may well be postponed until the full data are available and have been submitted to rigorous statistical analysis.

Riddle found that the first egg of each pair laid under normal conditions by pure species is generally 9–15 per cent. lighter than the second and contains less stored material. He observed similar differences between the earlier and later eggs of overworked series and between those laid by young and old birds respectively. He concluded from these results that there is a definite quantitative difference between male- and female-producing eggs. Since in birds the female is heterogametic this conclusion could be explained by supposing that a correlation existed between the size of the egg and the presence or absence in it of the Z-chromosome. This could be brought about by the directional influence of the chemical constitution and size of the egg on the reduction division, resulting in the retention in the egg or extrusion in the polar-body of the Z-chromosome. It is significant in this connection that in certain of Whitman's crosses all the male offspring were dark and all the female offspring were white. This suggests that these characters are sex-linked and that the sex-chromosome constitution is the normal ZZ♂ ZW♀. In other words, the ratio is modified by selective retention in or elimination from the eggs of the Z-chromosome, and not by reversal of the chromosome mechanism.

Seiler's [206] experimental work on insect eggs is relevant to this suggestion. He found that staleness and high temperature both resulted in the Z-chromosome of *Talæporia tubulosa* being retained in the egg more often than it was extruded in the polar body. It was extruded in the polar body more often than it was retained in the egg under normal conditions and under low temperatures. His results are summarised in Table XX, where male and female respectively indicate the retention or expulsion of the Z-chromosome of the egg as shown by cytological examination.

Table XX.

Conditions.	Numbers observed.	Per cent. retention of Z-chromosome.
Normal, 12°–16° C.	61 ♀ : 41 ♂	40·2
Staleness, 12°–16° C. . . .	101 ♀ : 146 ♂	59·1
Warmth, 30°–37° C. . . .	52 ♀ : 84 ♂	61·8
Cold, 3°–8·5° C.	49 ♀ : 32 ♂	39·5

Sex-reversal of Female Birds.—Abnormalities of sex in the barn-door fowl are comparatively common and are known to all poultry farmers as "crowing hens." Their recognition in ancient times is shown in the fourteenth-century French proverb :

> "C'est chose qui moult me deplaist,
> Quand poule parle et coq se taist."

This superstitious dislike is still common among the peasantry, and crowing-hens consequently are given "short shrift." Professor Butler has drawn my attention to an amusing anecdote of the treatment of one of these unfortunate birds in mediæval times, referred to in Chambers's *Book of Days* as follows : "At Basle in 1474 a cock was tried for having laid an egg. For the prosecution it was proved that cock's eggs were of inestimable value for mixing in certain magical preparations ; that a sorcerer would rather possess a cock's egg than be master of the philosopher's stone ; and that in pagan lands Satan employed witches to hatch such eggs from which proceeded animals most injurious to all of the Christian faith and race. The advocate for the defence admitted the facts of the case, but asked what evil animus had been proved against his client, what injury to beast or man had it effected ? Besides, the laying of the egg was an involuntary act and as such not punishable by law. If the crime of sorcery were imputed, the cock was innocent ; for there was no instance or record of Satan ever having made a compact with one of the brute creation. In reply the public prosecutor alleged that, though the devil did not make contracts with brutes, he sometimes entered into them ; and though the swine possessed

by devils, as mentioned in Scripture, were involuntary agents, yet they were punished by being caused to run down a steep place into the sea, and so perished. The pleadings in this case, even as recorded by Hammerlein, are voluminous ; suffice it to say that the cock was condemned to death, not as a cock but as a sorcerer or devil in the form of a cock, and was, with its egg, burned at the stake, with all the due form and solemnity of a judicial punishment."

The structural abnormalities included under the popular term of " crowing hen " generally conform to one of three categories : (*a*) Birds in which the gonads have been arrested in development or have been destroyed pathologically ; (*b*) hermaphrodite birds ; and (*c*) females transforming into males. We are not concerned with the first two categories in this chapter. The majority of the birds described as hermaphrodites were probably cases of sex-reversal, although a few, such as that of Hartman and Hamilton,[107] already referred to (Chapter X), were undoubtedly permanently hermaphrodite.

Hermaphroditism and sex-reversal in birds have been described chiefly in the game-birds (Gallinæ) and pigeons (Columbæ). This may be due in a great measure to the frequency with which fowl, pigeons, and pheasants come under observation. It is indicated, however, that sex is more unstable in these forms than in others, since cases are not known in ducks and geese where we might expect its detection.

Natural Sex-reversal.—Sex-reversal in birds was recognised first by Boring and Pearl, [22] in 1918, in dealing with a series of eight hermaphrodite fowl. They described birds which " were changing from a female to a male condition in respect of internal structure (gonads), external characters, and sex behaviour." Four of these birds had more or less degenerate ovaries with an incipient formation of sex-cords. The other four are remarkably clear cases of sex-reversal. One had a large coiled oviduct, similar to that of a laying hen, on the left and a small one on the right. This bird had a degenerate ovary without oocytes, but containing traces of degenerate follicles and also sex-cords, on the left. A large

testis was present on the right, in the tubules of which spermatogenesis was actively proceeding and motile spermatozoa were present. A second bird had a small oviduct and a large ovotestis on the left. Oocytes of various sizes were present in the ovarian regions and the spermatic tubules contained spermatogonia and spermatocytes in the contraction phase, but no spermatozoa. The other two had large oviducts and ovotestes on the left. Spermatogenesis was proceeding and sperms were present in both. The ovarian tissue was very degenerate and contained no oocytes in one case. The other was known to have laid and the gonad contained oocytes of all sizes. Crew [57] describes a similar series of seven fowls in process of sex-reversal and one which had transformed completely from a fertile female into a fertile male. The seven birds in process of reversal all had ovotestes on the left side in which the ovarian elements were more or less degenerate. Two of these had testes on the right, and exhibited active spermatogenesis and mature sperms in the gonads of both sides. The spermatic tissue in the others was in the form of sex-cords or tubules which did not exhibit spermatogenesis.

The case of complete reversal is unique and requires more detailed description. This bird was a Buff Orpington, which laid well until she was three years old, and was said to be the mother of chickens. She began to crow when $3\frac{1}{2}$ years old, and the comb and wattles commenced to develop like those of a cock. At this time she was obviously suffering from disease, but the development of the head furnishings continued and the spurs began to grow. The bird moulted and developed the plumage of a male. She had assumed all the external characters, with the exception of her female stance, and the habits of a male when $4\frac{1}{2}$ years old. She was then mated with a virgin Buff Orpington hen. This hen laid and incubated, ultimately hatching two eggs. The sex-reversed bird died when a little over five years old. Autopsy revealed advanced tuberculosis of the abdominal organs. A large tumour with a testis embedded in its dorsal side was situated in the left ovarian region. A testis was also present on the right. Each testis measured $3\frac{1}{2} \times 2$ cms. and was associated with a vas

deferens. A thin, straight oviduct was also present on the left side. The testes were found to be normal and functional, although not very active. Spermatogenesis was proceeding in the tubules and ripe spermatozoa were present in them and in the epididymis. The two chickens, of which this bird was the male parent, proved to be one male and the other female. This fact shows that the female fowl is heterogametic and supports the cytological evidence. Other cases of partial sex-reversal in fowl have been described (Berner [16], see also [87] and [187]). The three birds concerned all had a fairly well-developed oviduct and an ovotestis on the left side. They were hen-feathered but with head-furnishings similar to those of a cock. Two of these birds had a history of laying, but the history of the other was indefinite and its gonad contained no oocytes, although the ovarian nature of the stroma was unmistakable. The spermatic tissue in all three was in the form of sex cords or degenerate tubules without germ-cells. Another example is recorded in a pigeon,[33] which had a well-developed oviduct and a large testis on the left and an ovotestis on the right. The previous history was unknown, but the structure of the gonads demonstrated that the bird was in process of sex-reversal (Pl. XXII). The irregular contours of the gonads and the greater size of the left, together with the presence of an oviduct, showed that the bird was a female previously. The superficial position and degenerating condition of the ovarian portions, which contained oocytes, and the various stages of development (Figs. 24 and 25) of the spermatic tissue, which was in process of active formation and growth in many places, supported this conclusion. The spermatic tubules contained spermatogonia and spermatocytes exhibiting the typical contraction phases (Fig. 25). Spermatozoa were not present and the older tubules were distinctly degenerate.

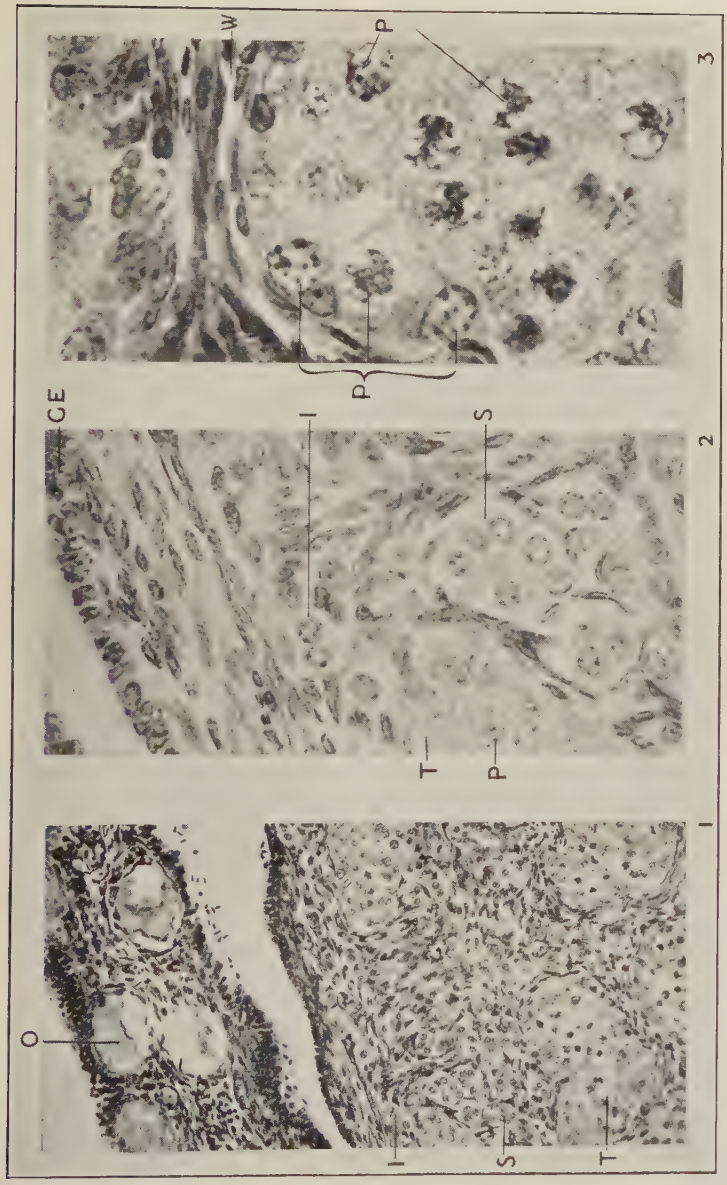
The majority of these cases of natural sex-reversal in birds were suffering from disease affecting the ovary, either neoplasms or tuberculosis. Some, however, showed no signs of a pathological condition. It is probable, therefore, that although the reversal may be initiated in most cases by pathological

PLATE XXII

Sex-reversal in a Pigeon

1. Portion of the ovotestis showing degenerating oocytes (O), islet cells (I), spermatic tubules (T), and various stages (S) in the formation of the spermatic tubules from the islet cells. × 200. 2. Higher magnification of part of Fig. 1 showing stages in the formation of the spermatic tubules in greater detail. The nuclei of the spermatic tubule can be seen to be in the prophases of the heterotypic division. GE, germinal epithelium. × 730. 3. Part of a well-formed spermatic tubule showing various prophase stages in the spermatocytes (P) and the wall of the tubule (W). × 1000. (After [33].)

PLATE XXII



destruction of the ovarian tissue, this explanation cannot be applied to other cases, as, for instance, that of the pigeon, which showed no traces of disease.

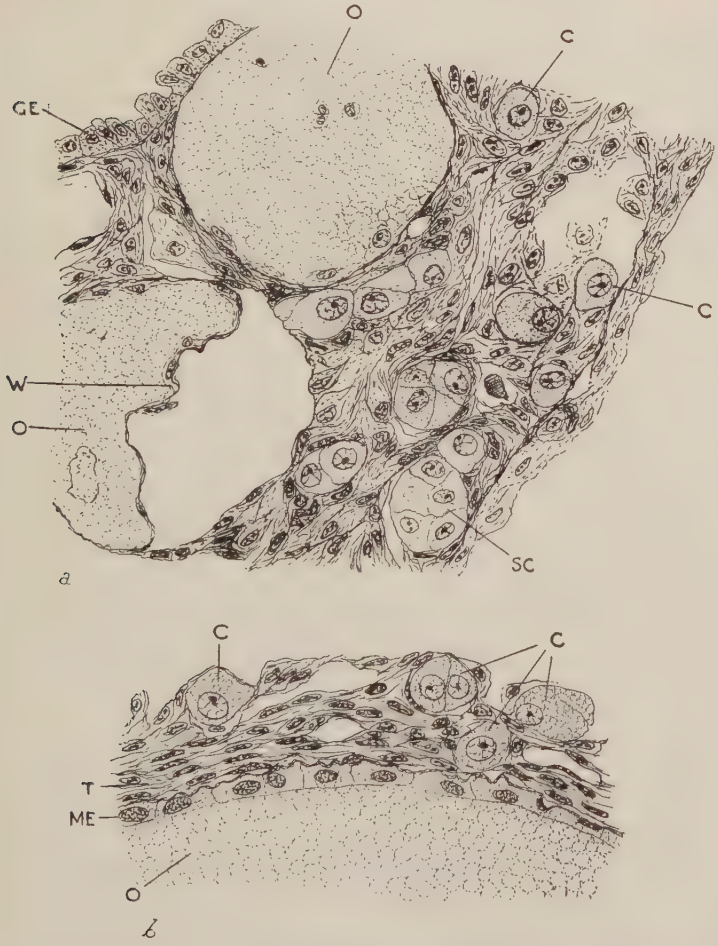


FIG. 24.—(*a*) Part of the ovarian portion of the ovotestis of a pigeon showing the germinal epithelium (GE), degenerating oocytes (O), and follicle wall (W). Islet cells are plentiful, occurring singly (C), and in small groups (SC). ×630. (*b*) Part of the wall of a nearly normal follicle from the same region showing the periphery of the oocyte (O), the follicular epithelium (ME), and the theca (T) with islet cells (C) in it. × 630. (From [33].)

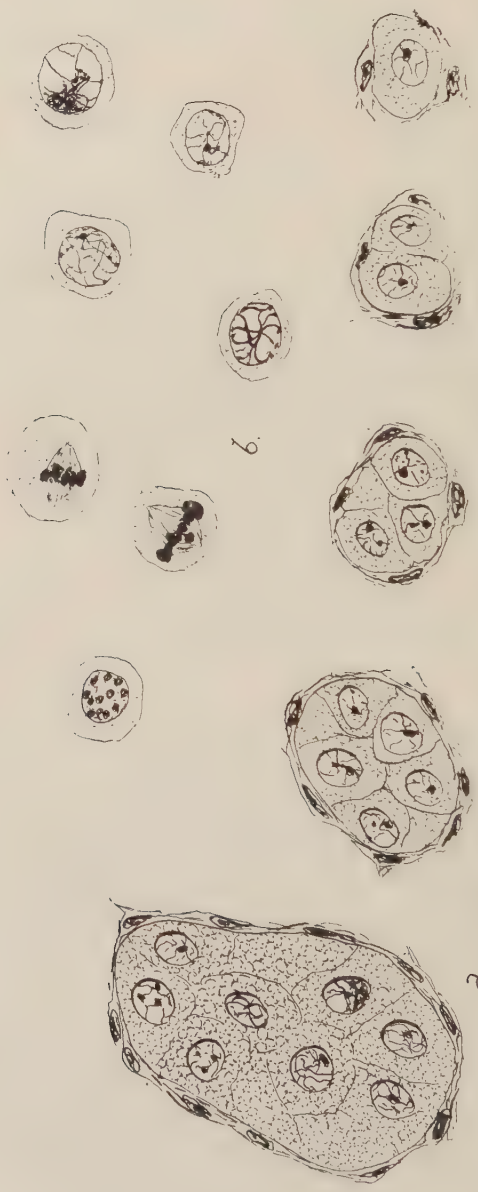


FIG. 25.—(*a*) Diagrammatic representation of five consecutive stages in the formation of sex-cords from the islet cells in the ovotestis of a pigeon. Note the prophase nuclei in some of the cells of the last stage. × 1600. (*b*) Spermatocytes exhibiting various stages of the maturation divisions from the spermatic tubules of the same pigeon. × 1100. (From [33].)

The left gonad was found to be an ovotestis in all except the pigeon, and the complete reversal described by Crew, in which the ovarian tissue had been entirely destroyed. The right gonad, on the other hand, when present was a testis, with the exception of the pigeon. This is explicable on the grounds that the right gonad in female birds is usually arrested in an indifferent condition and is not differentiated into an ovary. Consequently it would be expected to develop directly into a testis after sex-reversal. Occasionally this right gonad is definitely differentiated into an ovary, especially in pigeons, and may even be functional. It would then have to pass through an ovotestis stage like the left gonad before forming a testis. The pigeon described belonged apparently to this category.

Experimental Sex-reversal.—Sex-reversal in female fowl has been effected also by experimental means, and the various conditions of the right and left gonads, described above as occurring in nature, have been reproduced artificially. The results obtained may be summarised under three distinct headings : (*a*) development of the right gonad ; (*b*) regeneration of left gonad ; and (*c*) ovarian grafts.

Development of the Right Gonad.—Goodale [94] observed the development of a testis-like structure at the site of the rudimentary right gonad of hens which had had the left ovary removed previously. Benoit [14, 15] records two cases in which the right gonad developed into a testis which exhibited spermatogenesis. The left ovary was removed from one of these when it was twenty-six days old. The right gonad had developed when the bird was six months old. The left ovary of the other bird was removed when it was four days old. Testes were present on both sides when the bird was killed at the age of $9\frac{1}{2}$ months. The right testis was the larger and contained abnormal spermatozoa. The left testis was composed of cords similar to those of an embryonic testis.

Zawadowsky [251] observed the development of a testis on the right side in all the eight cases in which the left ovary had been completely removed. The right testis in six of these

birds, and in another in which the left ovary had been incompletely removed and had regenerated to form an immature testis, was composed of sex cords. The testis of one, named "Prima," 2½ years after the ovary had been removed, contained tubules exhibiting the early stages of spermatogenesis. The testis of the other, which had the left ovary removed ten months previously, exhibited spermatogenesis and active spermatozoa in some of the tubules. Finlay [80] and Greenwood [98] describe two cases of hypertrophy of the right gonad following left ovariotomy. A chick ovariotomised when two days old had a testis 0·5 cm. in diameter on the right side when killed fifty-two weeks after the operation. A lumen was present in most of the spermatic tubules, but there were no signs of spermatogenesis. Another chick ovariotomised when seven days old and killed forty-seven weeks later had an ovotestis measuring 1·3 × 0·3 cm. on the right side. The ovarian tissue in this gonad was normal and contained growing, as well as degenerating, oocytes. The spermatic tissue consisted of sex-cords and tubules, but there were no indications of spermatogenesis. The most extensive series of experiments on this problem are those recorded by Domm [67]. He removed the left ovary from sixty young hens. Two of these developed an ovary on the right side and all the others a testis. Fifty-six other birds regenerated a gonad on the left side as well as developing that on the right. One of these had an ovotestis on each side. The remaining fifty-five all had testes on the right, accompanied on the left by a testis in forty, by an ovotestis in one, and by an ovary in fourteen cases. Domm examined histologically nearly half of the right testes and found them to be composed of immature tubules which showed no signs of spermatogenesis. Domm [67] points out that the hypertrophy of the right gonad depends on the removal of the greater part of the left ovary and that it does not take place if a large mass of the ovary is left in. It is therefore to be considered as a true compensatory hypertrophy. Benoit,[14, 15] Zawadowsky,[251] Finlay,[80] and Domm [67] record in all 129 cases of hypertrophy of the right gonad in fowl. The sex of these gonads and of the left gonads in

those cases where it regenerated are shown in the accompanying table.

TABLE XXI.

No. of occurrences.	Right gonad.	Left gonad.
68	Testis.	No regeneration.
42	Testis.	Testis.
1	Testis.	Ovotestis.
14	Testis.	Ovary.
1	Ovotestis.	No regeneration.
1	Ovotestis.	Ovotestis.
2	Ovary.	No regeneration.
Total 129		

The right gonad developed into a testis in 125 or approximately 97 per cent. of the cases. It formed an ovotestis in two cases and an ovary in two. The occurrence of ovarian tissue on the right side in only 3 per cent. of the birds is of the same order as the normal differentiation of the right gonad into an ovary in fowl. It is therefore possible that the four birds in question can be accounted for by supposing that they possessed differentiated ovarian tissue on the right side at the time of ovariotomy, and it is not necessary to suppose that it was differentiated subsequently.

Regeneration of Left Gonad.—The left gonad regenerates always after partial ovariotomy and frequently after apparently complete ovariotomy. The regeneration in the latter case probably takes place from minute fragments which were not removed, but the possibility of regeneration after the removal of the entire gonad, such as has been demonstrated in mice,[188] cannot be denied. However this may be, the regenerated left gonad may be an ovary, an ovotestis, or a testis. Its nature appears to depend to a large extent on the mass of gonad from which the regeneration took place. The regenerate organ appears to be a normal ovary, as a rule, when a considerable portion of the original organ was left *in situ* at operation. Domm,[67] however, records the regeneration of all three types of gonads on the left side after apparently complete

removal of the original left ovary. He records the formation of a testis on the left, always accompanied by the development of one on the right also, in forty cases. Benoit [14, 15] and Zawadowsky [251] record similar cases. The regeneration of ovotestes on the left side have been recorded by Pézard, Sand, and Caridroit,[190] Greenwood [98] and Domm [67]. They may or may not be accompanied by a testis or ovotestis on the right side. The fact that a testis was present on the right in all the forty cases recorded by Domm in which a testis was regenerated on the left, supports the view that the latter was formed from a very small fragment of ovary, since the right gonad hypertrophies only in these circumstances.

The nature of the left gonad, if it regenerated, in the birds with hypertrophied right gonads referred to above, is shown in the third column of Table XXI. It is interesting to note that fourteen examples developed a testis on the right and a regenerate ovary on the left, a fact which will be referred to later.

Ovarian Grafts.—Ovarian grafts which have taken, whether implanted into the female from which they were taken, or into another fowl, male or female, frequently exhibit a development of sex-cords and spermatic tubules exactly similar to that observed in regenerate ovaries. Many examples of this phenomenon have been recorded by Caridroit,[48] Zawadowsky,[251] Greenwood,[98] and others. The tubules produced in regenerated or engrafted ovaries have not been observed so far to produce active spermatozoa or even to exhibit spermatogenesis, but their similarity to the testicular structures in the other cases of sex-reversal referred to in this chapter leaves their spermatic character beyond doubt. It may be expected with reason that further experiments will result in this form of sex-reversal being obtained in a more complete degree than heretofore. The cause of this transformation in engrafted and regenerating ovaries is probably the same in both cases. It has been pointed out that in the latter case the mass of ovarian tissue which effects the regeneration is probably an important factor; when this is small a testis or ovotestis is produced, and when large a normal ovary. Caridroit [48]

arrived at a similar conclusion in regard to the grafts, having found that sex-cords and tubules developed in the small grafts, while the large ones retained their normal ovarian character.

The Origin of Spermatic Tissue in Female Fowl.—The right ovary in birds is normally rudimentary, having been arrested in development at a very early stage. It appears, however, to be always present even in the adult. Brode[44] has shown that in the adult fowl it consists of medullary cords in the majority of cases. More rarely cortical tissue is also present and may even be differentiated into an ovary. Occasionally the right ovary has been known to be functional, especially in pigeons. The medullary cords persist throughout life in the rudimentary right ovaries of fowl in the form of tubules or solid cords, apparently devoid of germ-cells. Primordial germ-cells can be observed in them until about three weeks after hatching, when they all appear to degenerate. There can be little doubt that these medullary cords develop into the spermatic tubules if a testis develops on the right side after ovariotomy. This explanation is simple and obvious, since the medullary cords develop directly into the spermatic tubules in the normal male. They have persisted in the right gonad of a normal female in a condition approximately the same as that in an embryo at the time when sexual differentiation is about to take place, except for a certain amount of fatty infiltration.

The occasional development of an ovary or ovotestis on the right side after ovariotomy is accounted for by the presence of cortical tissue in small quantities in some birds. These cortical rudiments might be expected to develop into ovarian tissue when the gonad undergoes the compensatory hypertrophy consequent to ovariotomy.

The process of differentiation of spermatic tubules in differentiated ovarian tissue presents another problem, essentially the same whether it is on the left or, in rare cases, on both sides. This change probably results from retrogression of the ovary, due in nature to pathological or other causes, and in experimental conditions to partial ovariotomy or trans-

plantation. The spermatic tubules in these cases might be expected to originate from either of two sources : (*a*) from a new formation of sex-cords proliferated from the germinal epithelium, or (*b*) from the remnants of the medullary cords contained in the ovary. The former origin has been described in detail by Fell,[76] who demonstrated the stages in the formation of the sex-cords from the germinal epithelium covering the ovary, and their development into spermatic tubules. The latter origin presents more difficulties. The medullary cords persist as degenerate strands in the hilum of the normal ovary and give rise also to the large, clear " interstitial " or " luteal " cells forming small islets in the stroma and especially in the thecæ of the follicles, and possibly to other epithelial elements indistinguishable from those of cortical origin. Fell [76] and others suggested the possibility of some of these medullary elements in the stroma contributing to the formation of the new sex-cords, and noted the presence of large numbers of " luteal " cells in the gonads in process of transformation. Fell considered that these " luteal " cells were being formed from some of the sex-cords which degenerated. The sex-reversed pigeon referred to, however, affords a clear demonstration of the transformation of the islets of clear (" interstitial " or " luteal ") cells into spermatic tubules. All the stages in this process were observed in this bird and there was no evidence of any other origin of the sex-cords and tubules (Pl. XXII). It must therefore be concluded that spermatic tissue can be developed in the ovary of a bird either directly from the germinal epithelium or from the persistent remains of the medullary cords.

The origin of spermatogonia in sex-reversed fowl is not in accord with the theory of the continuity of the germ-plasm. The view that these are derived entirely from the primordial germ-cells which migrate into the germinal ridge during embryonic life is meaningless in this respect, unless it is also assumed that they remain undifferentiated into somatic cells. This latter assumption in the case of sex-reversed birds is not supported by any evidence. Although inadequate in this respect, it is not disproved by those cases in which the

spermatic tissue originates directly from the germinal epithelium or from the medullary cords composing the right gonad. It might still be maintained in these cases that the spermatogonia are derived from primordial germ-cells which have remained in the ovary in an undifferentiated condition, indistinguishable from the ordinary epithelial elements. The origin of the spermatogonia in the case of the pigeon described, however, provides a definite disproof of this view. This material affords no evidence as to whether the islet cells, which gave rise to the spermatogonia, originated from germ-cells contained in the medullary cords or from somatic cells. It is obvious, however, that the islet cells must be admitted to be differentiated. Fell [77] states that these cells are formed by a process of fatty degeneration, which resembles at first that described in the formation of adipose cells. The mitochondria multiply and transform into lipoid vacuoles. These lipoid vacuoles give rise to larger fat vacuoles which, unlike those in adipose cells, do not fuse. The origin of the islet cells from abortive medullary cords or atretic follicles supports the cytological evidence that they are formed by a process of degeneration. The islet cells of the pigeon were, in fact, prior to transforming into spermatogonia, differentiated, probably degenerate, ovarian elements. Their transformation into spermatogonia is, therefore, an amazing instance of redifferentiation and constitutes, theoretically, a true neo-formation of germ-cells during adult life.

It is an interesting fact that sex-reversal in young or adult birds is always in the direction of female into male. No instance of partial or complete transformation of a testis into an ovary is known in birds.

CHAPTER XIII

GYNANDROMORPHISM AND THE CONTROL OF THE SEXUAL CHARACTERS

HERMAPHRODITE birds occasionally occur in which the secondary sexual characters of one side of the body are male and those of the other side female. These rare abnormalities are known as gynandromorphs. This term has been applied to other types of hermaphroditism and intersexuality, but it is desirable that it should be used only in connection with this particular kind. True gynandromorphs occur also among insects, and many examples have been recorded in which a half, a quarter, or other fraction of the body exhibits only the characters of one sex while the remainder exhibits those of the opposite sex.

Gynandromorphism is of great theoretical importance and, in birds, is difficult to explain. The secondary sexual characters in vertebrates are known to be controlled by hormones produced by the gonads and circulating in the blood-stream to all parts of the body. Therefore the occurrence of the secondary sexual characters of both sexes on opposite sides of the body in gynandromorph birds is not compatible with this theory of hormone control unless it is qualified by other assumptions.

Gynandromorph insects present a simpler problem, since the secondary sexual characters in them are not controlled by the gonads but develop independently, presumably under the influence of the same genetic factors which determine the sex of the gonads.

The first section of this chapter will be devoted to a description of the few known examples of gynandromorph birds. The second will deal with some of the theories of the

genetic origin of gynandromorph insects and their application to birds. The third section will be reserved for a discussion of gynandromorph birds in the light of the hormone theory of the control of the secondary sexual characters.

Examples of Gynandromorph Birds.—Weber [225] described a chaffinch (*Fringilla cœlebs*) in which the right side had the full plumage of the adult male while the left side had the dull plumage of the female. The division between the two was in the middle line and was sharply defined. The arrangement of the internal organs corresponded to that of the plumage, in that a normal round testis 2 mm. in diameter was present on the right side and an ovary $3{\cdot}5 \times 2{\cdot}0$ mm. on the left.

Weber also quotes two similar cases recorded by Cabanis [47]. One was a bullfinch (*Pyrrhula vulgaris*) with male plumage on the right and female plumage on the left. The other was a specimen of *Colaptes mexicanus*, with the full male plumage on the left and female or juvenile plumage on the right. Unfortunately there is no record of the anatomy of either of these birds.

Poll [192] has described a beautiful example of gynandromorphism in a bullfinch. The right side of the body had the characteristic coloration of the male and the left side that of the female. The red plumage on the right side of the breast and the grey-brown on the left were in striking contrast and were clearly defined from each other in the middle line. There was some red on the axillary feathers on both sides. A small normal testis was present on the right side together with well-formed male efferent ducts. The spermatic tubules contained germ-cells and Sertoli cells. An ovary, larger than the testis, was present on the left side, but the female ducts were atrophic. The ovary contained many normal oocytes ranging from 23 μ to about 300 μ in diameter. The gonads were therefore both apparently normal, and the sex of the plumage corresponded with that of the gonad of the same side.

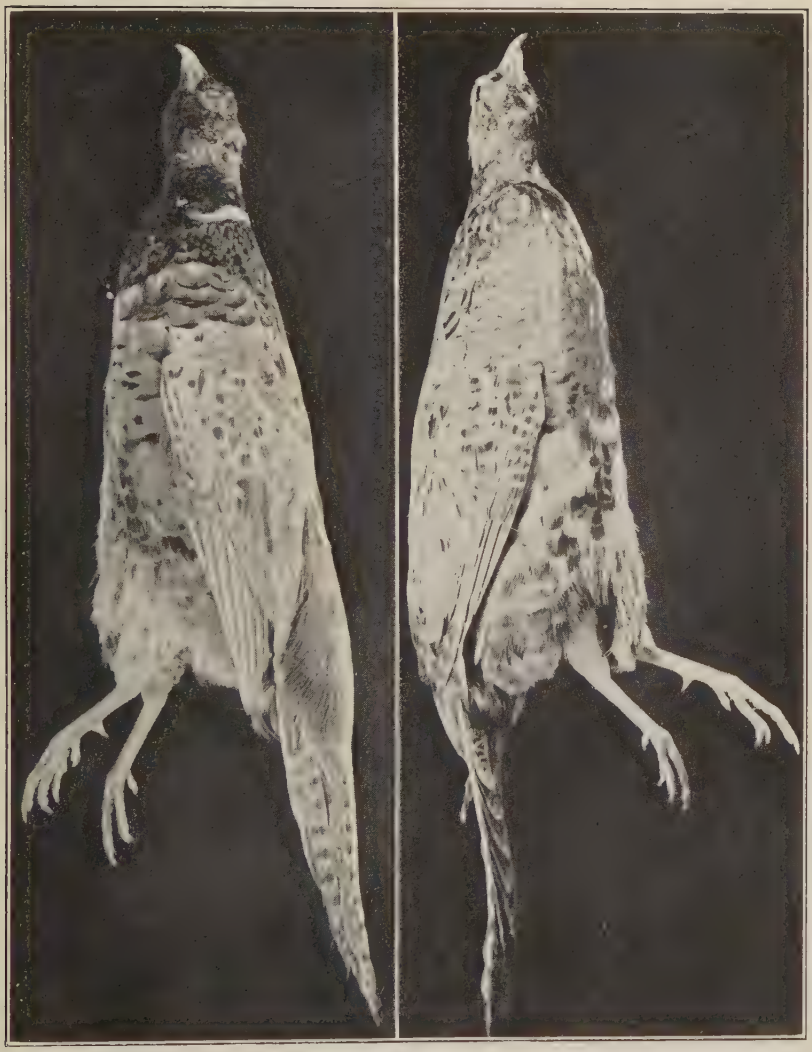
Bond [21] described a pheasant with a well-developed oviduct and an ovotestis on the left side, but no trace of a gonad on the right. The ovarian portion of the ovotestis appeared to be degenerating, while the testis portion appeared

to be in an active condition and to contain spermatozoa. The secondary sexual characters of the left side were predominantly male and those of the right side were female (Pl. XXIII). A large spur similar to that of a male was present on the left leg, while the right leg had only the rudimentary spur which is found in the hen pheasant. The bones of the left tarsus and phalanges were longer and thicker than the corresponding bones on the right. The red skin around the eye was much larger on the left than on the right. The head and neck had the blue-green irridescent feathers on both sides, but principally on the left. The white collar of the male Mongolian pheasant was present on the left side only. The wing primaries and most of the coverts were female, but a few male coverts appeared on the left wing. The breast feathers were black-tipped as in a male, but the rufous colour was deeper on the left. The tail coverts were male in character, especially on the left. The tail feathers were remarkable in that the outer vein of each feather exhibited the typical male marking, while the inner veins of the same feathers had the typical female colour pattern.

Macklin [150] has described a fowl which was probably a gynandromorph. It had a testis on the right side and an ovotestis and oviduct on the left. Oocytes were present in the ovarian portion and spermatogenesis was proceeding in the spermatic tubules, the lumena of which were crowded with spermatozoa. The skeleton was markedly asymmetrical, the bones of the right side being much larger than the corresponding bones on the left. The spur on the right leg was larger than that on the left. The plumage was largely that of the hen, but the feathers of the neck and of the tail were suggestive of those of the male. The comb was male in character.

There are thus at least three well-authenticated gynandromorph birds known, viz. Weber's Chaffinch, Poll's Bullfinch, and Bond's Pheasant. A partial explanation of the possible origin of these remarkable abnormalities is provided by gynandromorph insects.

Gynandromorphism in Insects.—Many examples of gynan-



1 2

BOND'S GYNANDROMORPH PHEASANT

1. Left or male side. 2. Right or female side. (From Bond [21].)

dromorph insects are known, chiefly belonging to the Hymenoptera, Diptera, and Lepidoptera. A classical example is that of Eugster's bees, investigated by Siebold [208] and more recently by Boveri [24] and Mehling[156]. These gynandromorphs appeared during several seasons in a hive of bees produced by an Italian queen and German drones.

The relative positions of the male and female parts in these bees, as in other insects, vary considerably, so that while in some one side is male and the other female, in others the anterior part is of one sex and the posterior of the other sex. Again, in others the major part of the body is of one sex, and only a limited region of the other sex. The male and female parts were, in fact, fitted together in various mosaic patterns. Boveri found that in these gynandromorphs all the male parts had the characters of the Italian race only, while the female parts had the hybrid characters of the German and Italian races. This fact is significant, since drone bees are normally produced from parthenogenetic bees and consequently inherit the characters of the mother only, while females are produced from fertilised eggs and inherit the characters of both parents. Boveri therefore suggested that these gynandromorphs may have been produced by abnormalities in fertilisation, resulting in some of the blastomeres developing parthenogenetically, while the others developed with the fused germ-nuclei. This condition would result if one or more cleavages took place before the male pronucleus fused with the female pronucleus. That blastomere in which the fusion was effected, which was in fact fertilised, would give rise to female parts, while the others would give rise parthenogenetically to male parts. This explanation appears probable in view of the inheritance of the male parts being purely Italian, while the female parts were hybrid.

Similar gynandromorphs in Drosophila are well known and have been investigated by Morgan and Bridges [159]. Analysis of the inheritance of somatic and sex-linked characters in some of these showed that the former were not concerned while the latter were. When, for instance, a dominant sex-linked gene and its recessive allelomorph were concerned, the

female parts showed the dominant character while the male parts showed the recessive. This suggests that the female parts contained both X-chromosomes, while the male parts contained only the X-chromosomes bearing the recessive character. It was suggested, therefore, that these gynandromorphs arose from genetic females (XX) in which one X-chromosome was eliminated at one of the segmentation divisions. When this happened at the first cleavage one blastomere was left with both (XX♀), while the other had only one (X♂), and a bilateral gynandromorph resulted. When it took place at any later division the resulting gynandromorph would be predominantly female and the extent of the male portion would depend on the stage of segmentation at which the elimination of the X-chromosome occurred.

This theory is supported by the fact that gynandromorphs of Drosophila are either approximately half male and half female, or else predominantly female.

Goldschmidt and Katsuki [93, 123] have described a number of gynandromorph silkworms which occurred regularly in certain strains. Breeding analyses showed that they were caused by the presence of a recessive gene and were consequently genetic. The theoretical explanation of their origin required the *a priori* assumption that two nuclei were present in the eggs and participated in development. Consequently a cytological investigation of the phenomena of maturation and fertilisation was carried out [123] to test this assumption.

The nucleus of the normal egg at the time of maturation is situated near the periphery at one pole of the egg in a cone of polar cytoplasm, free from yolk. The first maturation spindle is perpendicular to the surface of the egg, and division results in the formation of the first polar nucleus and the secondary oocyte nucleus. The polar nucleus lies between the oocyte nucleus and the periphery. Both nuclei undergo a second division simultaneously, the axes of their spindles being perpendicular to the surface of the egg. This results in the formation of four nuclei in a row in this axis. These are numbered 1, 2, 3, and 4 from the periphery inwards. Nos. 1 and 2 are the derivatives of the first polar nucleus ; No. 3 is

the second polar nucleus ; and No. 4 is the mature female pronucleus. The female pronucleus and the second polar nucleus are equal in size and are larger than the nuclei arising from the first polar body. The latter are unequal in size, and No. 1 is slower than No. 2 in passing from the telophase to the resting stage. One, two, or three sperms usually enter the egg at fertilisation. The male pronuclei to which they give rise lie deeper in the cytoplasm than the female pronucleus, and are also distinguishable by their denser staining and by the presence of the aster around each. The female pronucleus normally migrates to the nearest male pronucleus and fuses with it. At the same time Nos. 2 and 3, and possibly No. 1, of the polar nuclei fuse together to form a single nucleus which may undergo mitoses and persists in the egg for a considerable time. Normally it finally undergoes fragmentation.

An abnormal phenomenon was observed in a small percentage of the eggs in which the male and female pronuclei fused normally. The fused second and third, however, migrated into the deeper layers of the cytoplasm at the same time and took up a position symmetrical to the fused pronuclei. They developed an aster at the same time and were apparently preparing to participate in cleavage (Pl. XXIV, Fig. 1). The bi-nucleate eggs produced in this way obviously provided the conditions requisite for the explanation of the development of the gynandromorph, for the fused pronuclei would give rise to the nuclei of one half of the body and the fused polar nuclei to those of the other half. The chromosomal constitution of these nuclei will depend, since the female is heterogametic, on whether the first or second maturation division is reductional for the sex-chromosomes. The various possibilities are represented in Table XXII.

TABLE XXII.

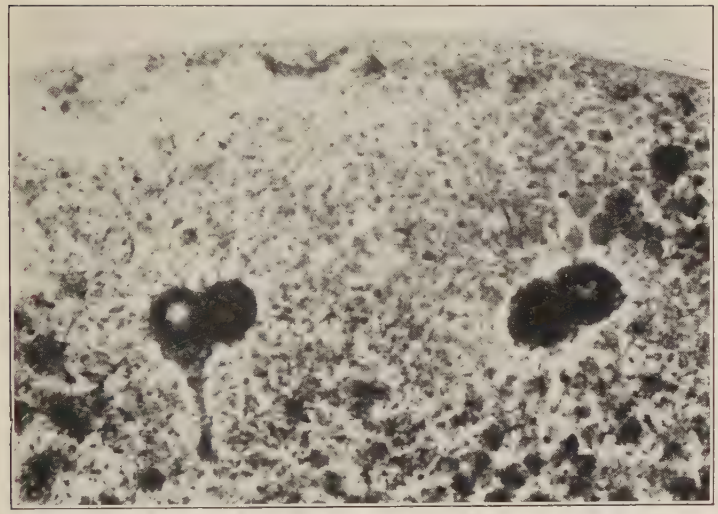
	Pre-reduction.		Post-reduction.			
1st polar nucleus { 1	Y	X	X	Y	X	Y
{ 2	Y } ♀	X } ♀	Y } ♀	X } ♂	Y } Non-	X } ♀
2nd polar nucleus 3	X }	Y }	X }	X }	Y } viable	Y }
Female pronucleus 4	X } ♂	Y } ♀	Y } ♀	Y } ♀	X } ♂	X } ♂
Male pronucleus	X }	X }	X }	X }	X }	X }

PLATE XXIV

Gynandromorphism in Lepidoptera

1. Photomicrograph of periphery of a fertilised egg of the silkworm moth which shows the manner of origin of the two nuclei. The male and female pronuclei are on the left, and the polar nuclei, about to fuse, are on the right. Each pair of nuclei is surrounded by an aster and both are behaving in a similar manner. 2. A complete bilateral gynandromorph of Papilio dardanus. Approx. nat. size. (Fig. 1 from Goldschmidt and Katsuki [93]. Fig. 2 from Poulton [195].)

PLATE XXIV



1

2

It can be seen from this table that with pre-reduction 50 per cent. and with post-reduction 66 per cent. of those cases in which the fused polar nuclei participated in development would give rise to lateral gynandromorphs.

These three explanations of the manner in which gynandromorphs arise in Bees, Drosophila, and the Silkworm are similar in that they all postulate that two nuclei, one XY or XO, and the other XX, take part in development. The male and female portions of the gynandromorphs differ, in fact, in the chromosomal constitution of the nuclei of their constituent cells. The details of the phenomena which result in this condition differ in each case, but the result in all is essentially the same. Moreover, the evidence, both genetical and cytological, in favour of this explanation of gynandromorph insects is very convincing.

It might be expected that abnormalities in the distribution of the autosomes similar to those of the sex-chromosomes in gynandromorphs would occur occasionally and would produce individuals asymmetrical as regards one or more somatic characters. Such abnormalities have actually been observed in insects, and a fowl described by Crew [59] probably belongs to the same class. Bilateral size differences have been recorded also in man and other mammals. They support the chromosomal interpretation of true gynandromorphs in so far as they show that the autosomes may exhibit comparable abnormalities.

Poulton [195] has recently drawn attention to some abnormal butterflies which arose from pupæ which had been subjected to mechanical shock by van Someren. One of these was a true lateral gynandromorph (Pl. XXIV, Fig. 2) and the others exhibited mosaic characters. It was suggested that these were the result of the shock administered to the pupæ. Their origin requires further investigation, but the author is inclined to share the view expressed to him by Prof. Huxley that only one (Pl. XXIV, Fig. 2) was an undoubted gynandromorph and that its appearance in the experimental culture may well have been purely fortuitous.

The explanation of gynandromorph insects in terms of a

different sex-chromosome constitution of the male and female parts of the body can be applied to gynandromorph birds. This explanation of the origin of gynandromorph birds is, however, incomplete in itself and requires further assumptions. The difficulty lies in the fact that the sexual characters in birds are not the direct local expression of genetic constitution of the tissues, as in insects, but are controlled by the gonads. It is necessary, therefore, to consider the nature of this essential difference.

Gynandromorphism and the Hormone Theory.— The most puzzling aspect of gynandromorph birds is that of the appearance of the secondary sexual characters of both sexes in an individual. The secondary sexual characters in birds, as in other vertebrates, are known to be developed under the influence of hormones. These hormones are produced in the gonads and circulate in the blood, thus reaching all parts of the body. They have not, up to the present, been successfully extracted from the tissues or obtained in a form in which they can be tested experimentally by injection into gonadectomised animals. The evidence for their existence is, nevertheless, extensive and convincing. It has been shown, for instance, that the secondary sexual characters in gonadectomised birds fail to develop normally and give rise to the characters of the capon. These characters are essentially the same in a gonadectomised male as in a gonadectomised female. Some of these characters, such as bone-size, can obviously only be affected by gonadectomy taking place before development is completed. Others, such as plumage, can be effected during adult life. Thus an adult hen will not develop the stance and configuration of a capon after ovariotomy, but will develop the plumage.

Successful grafting of parts of the gonad at the time of operation prevents this assumption of the capon characters. This shows that the position and innervation of the gonad tissue is irrelevant and that the blood-stream affords the means by which the hormones reach and affect the tissues. Successful grafting of either ovary or testis into a gonadectomised bird of either sex results in the assumption of the secondary sexual

characters of the same sex as the graft. This shows that the tissues of either sex are capable of producing the secondary sexual characters of the other sex under the influence of the appropriate hormones. It is clear, then, that the secondary sexual characters in birds are determined normally by sex-hormones, produced in the gonads, circulating in the blood-stream to all parts of the body. Similar results have been obtained with many mammals and other vertebrates. Somatic characters, on the other hand, which are common to both sexes, are not influenced by these hormones. They are apparently the expression of the genetic constitution of the tissues and are controlled locally in the tissues. This distinction is clearly shown by the skin-grafts in fowl recently described by Danforth and Foster [63]. These authors employed chicks of several clearly defined breeds and grafted pieces of skin from one to the other. A good proportion of these skin grafts took and subsequently developed feathers. It was found that the plumage characters on the grafts, even to the finest details of shade and texture, conformed exactly to those of the donor's breed and were uninfluenced by the breed of the host. It is clear, therefore, that these characters were the direct local expression of the genetic constitution of the skin graft and were not controlled by hormones in the host. The secondary sexual characters of the plumage on the skin grafts, on the other hand, were those belonging to the sex of the host, irrespective of the sex of the donor. Thus a graft of skin from a Rhode Island Red female into a White Leghorn male developed the plumage of a Rhode Island Red male. They developed feathers such as would be produced by an individual representing the breed of the donor and the sex of the host. This experiment has the advantage that the endocrine balance of the bird is not upset by gonadectomy or gonad transplantation, and the conditions in the graft are therefore as nearly normal as possible. The result confirms the view that the secondary sexual characters are controlled by sex-hormones.

The problem is : how do the secondary sexual characters of both sexes develop in gynandromorph birds, and why are

they limited to opposite sides of the body and clearly defined from each other in the middle line? It is apparent that both male and female sex-hormones are probably present in these birds, since both testis and ovary were developed. The problem then reduces itself to why the male hormone controlled the secondary sexual characters of one side and the female hormone those of the other side? Obviously the vascular system supplies no mechanism which could distribute either hormone to one side only. It must be presumed that both hormones were equally distributed to both sides. The only plausible explanation appears to be that the tissues of the two sides reacted differently, those on one side being more readily stimulated by the male, and those on the other by the female hormone. This difference must lie in a different genetic constitution of the cells of the two sides. This explanation has the merits of bringing gynandromorph birds into line with insects by admitting of the theory that they arise from abnormalities, at fertilisation or the first cleavage, which result in one blastomere containing $2n$+X-chromosomes (♀) and the other $2n$+2X (♂). Gynandromorph birds should be, therefore, genetic males (homozygous) in which the cells of half the body have one X-chromosome missing. It should be remembered, however, that although this explanation would account for the observed condition in gynandromorph birds, there is no confirmatory evidence from other sources that tissues of male constitution are more sensitive to stimulation by the male hormone than tissues of female constitution, or *vice versâ*. Moreover, it is difficult to explain the bilateral arrangement of the markings on the individual tail feathers of Bond's pheasant on this basis.

The Influence of Hormones on the Development of the Gonads.—Hormones produced by the gonads control the development of the accessory sexual organs in vertebrates in a similar manner to the secondary sexual characters. The evidence for this rests on extirpation and transplantation of the gonads. These hormones are necessary for the development of accessory organs in the young animal and to a less extent for their maintenance in the adult. There is no reason

to suppose that the hormones controlling the accessory organs are distinct from those which determine the secondary characters. The subject is, however, beyond the scope of this book and has been dealt with at length by Lipschütz.

There is also some evidence that the factors determining the differentiation of the gonads themselves into ovaries or testes are in the nature of hormones. They are presumably distinct from those which control the accessory organs and secondary characters. The evidence for these hormones that control the differentiation of the gonads is not extensive, and their existence is more or less hypothetical. Their presence is, however, indicated by the occurrence of Free-martin Cattle. These intersexes occur only as co-twins with bull-calves. They are females which exhibit various grades of intersexuality and are always sterile. The more extreme cases exhibit modifications of the ovaries into organs resembling undescended testes, and all show modification of the accessory sexual organs. Their origin has been studied by Keller and Tandler [124] and by Lillie [138, 139] and his pupils. They never occur in twins which are both of the same sex, but only as co-twins with normal males. The majority of females which are twins with normal males appear to be free-martins. They are associated with an early chorionic fusion and anastomoses of the placental blood vessels of the two embryos. Apparently this results in the hormones from the male twin, circulating in the blood of both, upsetting the normal development of the female twin and producing the structural modifications characteristic of the free-martin.

Many attempts have been made to reproduce these results experimentally by operative union of two young animals or embryos, and subsequent examination of the sexual organs of the *parabiotic* twins so produced. Such experiments were first performed on young rats by Morpurgo [160] and have been repeated since by several investigators. These experiments, however, did not lead to the modification of the sexual organs in any of the animals. This result is not surprising, since the differentiation of sex in mammals is completed before birth.

Burns [46] carried out a series of experiments on parabiosis in Amblystoma. The embryos were joined when in the tail-bud stage. Eighty pairs lived until sex could be determined, and of these forty-four were both males and thirty-six both females. It was inferred that one individual in each male-female pair had undergone complete sex-reversal. The mortality was so great (over 70 per cent.) that it may have eliminated all pairs combining the two sexes. The results are therefore unconvincing. Witschi [246] carried out a similar series of experiments on the American wood-frog (*R. sylvatica*). The embryos were joined at the age of fifty to seventy hours, shortly after the medullary tube had closed. Fifty-six pairs were reared successfully until after the period of sex-differentiation. Among the total of 112 individuals fifty-nine were males and fifty-three were females, eleven of which were in process of transformation into males. These were arranged in sixteen pairs of males, thirteen pairs of females, and twenty-seven pairs of male-females. All the sex-reversing females were in the last-mentioned group. Among 196 controls there were 100 ♀ : 96 ♂, none of the females showing signs of sex-reversal. This approximates to a 1 : 1 ratio on the basis of which one might expect the fifty-six pairs of parabiotic twins to be arranged as 14 ♂♂ : 28 ♂♀ : 14 ♀♀. The actual results approximate very closely to this expectation. It is clear, then, that no early sex-reversal has taken place in the parabiotic twins. The fact that eleven of the twenty-seven females united to males were in process of sex-reversal after the gonads had differentiated into ovaries, while none of the females among the controls exhibited sex-reversal, appears to show that the union with the male tends to bring about sex-reversal in the ovaries after they have differentiated as such.

Somewhat similar experiments have been performed on chick embryos by grafting bits of gonads on to the chorio-allantoic membrane. Minoura [157] reported abnormalities in the sexual organs of female chick embryos on which the grafts were of testes. Greenwood [97] and Willier [229], however, failed to confirm these results.

There is thus no experimental evidence of the testis exerting

an influence on the ovary in parabiotic rats. The evidence in birds has been contradicted and the evidence in Amphibia, though positive, is not well established or extensive. It is possible, however, that further experiments may lead to more convincing results.

CHAPTER XIV

CONCLUSIONS

The conclusions which can be drawn from the material presented in this book group themselves naturally under two heads: (*a*) The evolution of the gonads, and (*b*) the determination of sex. These two aspects of the biology of sex are distinct; the one is phylogenetic and the other ontogenetic. The results bearing on both are interwoven and therefore could not be separated conveniently for descriptive purposes. It is the purpose of this concluding chapter to summarise these results under their proper headings and to attempt to assimilate them into a general scheme.

The Evolution of the Gonads in Vertebrates.—The evidence appears to justify the conclusion that the primitive ancestor of the vertebrates had a number of pairs of gonads arranged segmentally. These gonads were without ducts, and the mature germ-cells were liberated from them by rupture and escaped into the cœlom. The sexes probably were distinct. The spermatozoa were flagellate and motile. The ova were small and microlecithal. Both ova and spermatozoa were produced in enormous numbers, and fertilisation was effected externally in the sea-water. Moreover, it is probable that secondary sexual characters were entirely absent and that the gonads had no endocrine function. The germ-cells must have escaped from the cœlom to the exterior either by rupture of the body wall or else through a cœlomipore which perforated the body wall.

The conditions existing in Amphioxus correspond exactly to these hypothetical requirements, and this form must therefore be considered, in so far as the reproductive system is

concerned, as not far removed from the primitive ancestor of the vertebrates.

The Cyclostomes do not exhibit much advance from this primitive condition. The germ-cells in both sexes escape into the cœlom and reach the exterior through a pore in the body-wall. Fertilisation is external and purely fortuitous. There are no secondary sexual characters and therefore little evidence that the gonads have assumed an endocrine function. The segmental arrangement of the gonads is lost, however, and little or no trace of its former existence can be detected. This is true, also, of all higher vertebrates, although there are indications in the Amphibia of the ancestral segmental arrangement of the gonads. The eggs of the Cyclostomes, however, exhibit a distinct advance, for they are larger and more yolk-laden. This is the first indication of that evolutionary trend which has resulted in the development of the macrolecithal eggs, so typical of the vertebrates, which has attained its climax in the Elasmobranchs and Sauropsida.

The development of the macrolecithal ovum is of fundamental importance and has had a far-reaching effect upon the evolution of the vertebrates. It provides the key to the proper understanding of the evolution of their reproductive organs.

The presumable cause underlying the development of the macrolecithal ova in vertebrates was the need of producing the young at a more advanced stage of development when hatched. Its result was the profound modification, produced directly or indirectly, of the reproductive organs of both sexes.

The production of macrolecithal ova involved in the female the development of ovarian follicles capable of conveying sufficient nutriment to the growing oocyte to meet its relatively huge demands. The elaborate and highly differentiated follicles of the Elasmobranchs and Sauropsida were evolved in consequence from the primitive follicles, each composed of a single layer of epithelial cells investing the oocyte, found in lower forms.

The production of large ova also involved the development of ducts capable of conveying them to the exterior, since the

occurrence of a rupture, or the presence of a simple pore, in the body-wall, sufficiently large to allow of the escape from the cœlom of these ova, would be a serious disadvantage. The oviducts were evolved from the Müllerian ducts to meet this requirement. The morphological origin of the Müllerian ducts is, however, uncertain. They develop as peritoneal funnels, one or more of which fuse together into a single ostium abdominale tubæ on each side. The tip of the ostium grows back beneath the peritoneum of the genital ridge as the Müllerian duct and acquires an opening into the cloaca. Brachet [26] suggests that the funnels which form the ostia may represent pronephric nephrostomes which have acquired the function of genital ducts in the female and have persisted as such. This interesting suggestion is based on very slender evidence, but it is difficult to explain the origin of the oviducts on any other hypothesis.

The subsequent modification of the oviducts to secrete albumen, thus still further increasing the reserve of nutriment for the development of the embryo, and to form a membranous or calcareous shell for its protection, is sufficiently obvious.

The efferent ducts in the males of Elasmobranchs, Amphibians, and Amniotes are constituted by the anterior mesonephric tubules and the Wolffian duct, which have assumed the function of genital ducts as a secondary modification. The sperms are liberated into the cœlomic cavity in primitive vertebrates. Brachet [26] suggests that in such forms the mesonephric nephrostomes furnished a means of escape. It is possible to imagine that, as the nephrostomes became cut off from the cœlom, connections between them and the spermatic tubules persisted and constituted the rete testis of higher forms. This explanation of the origin of the urogenital connection is attractive, especially when it is remembered that a condition still more primitive than that postulated actually exists in Cyclostomes. Whatever the origin of the urogenital connection the sperms undoubtedly escaped from the testes into the cœlom in primitive forms, as they do in Amphioxus and the Cyclostomes. Moreover, this primitive condition is retained in the females of all vertebrates. The

urogenital connection in the male is thus a secondary modification.

The development of genital ducts in the male and female provided the necessary antecedent for the adoption of some method by which the spermatozoa could be introduced into the female and fertilise the ova before they were laid. The advantages of internal fertilisation are obvious, since it provides for the fertilisation of a much higher percentage of all eggs and at the same time a greatly reduced expenditure of spermatozoa. External fertilisation, which is fortuitous, involves prodigal wastage of both ova and spermatozoa. Decreased wastage of ova is especially necessary in macrolecithal forms which can produce only relatively few eggs. This requirement has been met by the parallel evolution in different groups of copulatory organs in some form or another. Thus Elasmobranchs and some Teleosts have developed claspers on the anal fins of the male, which serve to convey the sperms from the male to the cloaca of the female. Fertilisation is external in many Teleosts and many Amphibians. However, the coupling of Anurans during laying serves a similar purpose to the introduction of the spermatozoa into the female genital tract, since it results in the fertilisation of the ova as they are laid. Among the reptiles paired copulatory organs are developed on the ventral wall of the cloaca in male lizards and snakes. Tortoises, crocodiles, and many birds also have a copulatory organ in the form of a single median penis, with a groove for the conveyance of the seminal fluid, developed in the floor of the cloaca. The well-developed penis of the mammals is developed from this latter type, and intermediate stages in its evolution are provided by the Monotremes and Marsupials. It is noteworthy in this connection that while internal fertilisation is an advantageous adaptation in aquatic vertebrates, it is an essential in terrestrial forms and takes place invariably in the Sauropsida and Mammalia.

The Teleosts in several respects do not fit into this evolutionary scheme. Their eggs are not very large, as a rule, and the arrangement of the genital ducts is very different from that found in Elasmobranchs and all higher vertebrates. The

Elasmobranchs, in so far as the reproductive organs are concerned, appear to be primitive and not far removed from the line of direct ancestry of the land vertebrates. The Teleosts, on the other hand, do not exhibit a primitive arrangement of the reproductive organs. They have undergone profound secondary modification and are, in so far as the gonads and gonaducts are concerned, a divergent group. This conclusion is supported by even the detailed structure of the gametes, both the spermatozoa and ova, which do not occupy an intermediate position between those of the Elasmobranchs and Amphibians. The study of their reproductive systems alone leads to the conclusion that they diverged at an early stage in their evolution from the ancestral stock which was destined to give rise to the land vertebrates.

Accessory sexual organs and secondary sexual characters are found first in the fishes. Moreover, it is known from cases of sex-reversal that the gonads control their development in Teleosts. There is, therefore, no reason for supposing that the gonads had any endocrine function in more primitive forms. This function was, in all probability, only developed with the evolution of different genital ducts in the male and in the female. It might be supposed, for example, that the oviducts, as they developed, acquired a sensitivity to some product of the ovarian metabolism which gradually became a necessary stimulant for their complete development. A by-product, possibly even a waste product, of metabolism of either the ovary or of the testis would become, in this way, a hormone. It is probable that the gonads, and, indeed, other glands of internal secretion, first acquired their endocrine function in some such simple manner.

The appearance of the mammals marked a profound change in the trend of development of the reproductive system. The salient feature, as has been pointed out, in the evolution of sex in other vertebrates, was the tendency to produce increasingly large and yolk-laden ova. This was due to the premium set upon the production of young that were already relatively advanced in development when hatched. The adaptation of the mammals to gestate their young in the

uterus and, further, to nourish them after birth by the secretion of milk, achieved the same result in a more efficient manner. The necessity of producing heavily yolk-laden ova was obviated and a secondary reduction in the size of the ova resulted. The characteristic modifications of the female genitalia of mammals are associated with these phenomena. The modification of the oviducts to form Fallopian tubes, uteri, and vagina, and the progressive development of the mammary glands from sebacious glands is well known already. The significance of the modifications of the ovaries associated with these phenomena requires further attention.

The Monotremes exhibit the first stages in the reduction of the size of the ova consequent on the assumption of gestation and lactation. The ova, compared to those of other mammals, are still comparatively large. This is due to the fact that the eggs are laid before they are hatched and that, in consequence, they still require a considerable reserve of nutriment. They do, however, absorb a considerable amount of nutriment from the oviduct which also secretes a layer of albumen and a leathery egg-shell around them. This is shown by the fact that the oocyte when ovulated only measures 4·5 mm. in diameter, while the new-laid egg is ovate, being about 17 mm. long by 15 mm. broad. The chief difference between the ovaries of Monotremes and those of the Sauropsida is to be found in the structure of the follicles and in the corpora lutea. The mature Monotreme follicle possesses a well-defined theca interna between the theca externa and the membrana granulosa. Correlated with this is the formation of a definite corpus luteum in the ruptured follicle. Moreover, the cells of all three layers of the follicle contribute to the formation of the corpus luteum. This suggests that the theca interna has been developed with the definite function of taking part in the formation of the corpus luteum. The first appearance of a definite corpus luteum in Monotremes is significant, since it is known to be, in many mammals, an endocrine organ controlling gestation and lactation. It is represented in the Sauropsida merely by a degenerating mass of cells formed by the retrogression of the ruptured follicle. Apparently this

degenerating tissue assumed the control of gestation and lactation and evolved into the highly organised mammalian corpus luteum. Probably the functions of gestation and mammary activity gradually came to depend, through constant association, on the presence in the blood of a substance peculiar to the metabolism of this retrogressing tissue. Through this chance association and ultimate dependence the waste product became a hormone. Once this was established the subsequent organisation of the tissue occupying the discharged follicle into the glandular corpus luteum is not difficult to imagine.

The final stage in the reduction of the deutoplasmic content and, consequently, of the size of the ova is observable in the Marsupial and Eutherian mammals. In them the amount of yolk in the cytoplasm of the ova is small, and holoblastic segmentation takes place in consequence. The amount of deutoplasm present in the mature ovum is reduced still further by extrusion from the ovum at the time when the first cleavage is effected. This process of deutoplasmolysis completes the reduction of the yolk content of the egg and facilitates holoblastic segmentation.

The mammals with microlecithal ova are also characterised by the development of the true Graafian follicle with a large antrum filled with liquor folliculi. It has been indicated that this modification of the follicle is probably necessary to provide for the formation of the corpus luteum and to effect ovulation. A much smaller follicle would be quite sufficient for the requirements of the growing oocyte. The small oocyte, on the other hand, might not be able to burst through the superficial tissues of the ovary, but the large, rapidly growing Graafian follicle provides a mechanism capable of rupturing the overlying tissues and effecting ovulation. Moreover, when ruptured, the large, hollow Graafian follicle provides a sufficient area for the formation of the corpus luteum, which could not be effected so rapidly if the follicle was small.

Regarding the origin of the germ-cells in vertebrates, it must be admitted that many points are still unsettled. However, there can be little doubt that the primordial germ-cells

either do give rise to definitive germ-cells or else did so in some ancestral form. Moreover, it seems certain that they are formed at a very early stage of embryogenesis in the extra embryonic endoderm of the yolk-sac and that they subsequently migrate into the developing germinal ridges. The problem of whether any or all of the definitive germ-cells are derived from the primordial germ-cells is still in doubt. It seems probable, however, that, at all events in special circumstances, secondary germ-cells are formed. The weight of evidence, especially that based on sex-reversal and gonad regeneration, appears to favour this conclusion. It may be supposed, if the primordial germ-cells are abortive, that they represent the functional germ-cells of more primitive forms in which sexual maturity was attained very early in life. This would account in part for their early origin in the embryo, and the subsequent postponement of sexual maturity might have made desirable the formation of secondary germ-cells. The gradual postponement of sexual maturity may be invoked to account for the occurrence of the two or three successive epithelial proliferations which give rise to the ovarian cortex in the higher vertebrates, for it seems likely that the medullary cords alone give rise to the ovaries of primitive vertebrates, as they do to the testes of even birds and mammals.

The Determination of Sex.—It is clear that the essential concepts of the sex-chromosome theory are firmly established. They are supported by an immense body of evidence, both genetical and cytological. The sex-chromosomes, on account of their unequal distribution in the two sexes, provide the mechanism which normally determines whether the fertilised ovum will develop into a male or into a female. It seems certain, however, that the sex-chromosomes are only one of a complex of genetical factors involved in sex-determination. The other factors appear to be distributed equally to both sexes and are probably borne by the autosomes or possibly even in the cytoplasm. The presence of one or two X-chromosomes, therefore, normally tips up the balance of the whole complex in either the male or the female direction. They determine sex only in the sense that they hold the

balance. Sex is the result of the interaction of a number of inherited factors or genes and of the environment. It is thus essentially similar to any other character. It is possible that any of these factors, hereditary or environmental, might be so modified in certain circumstances as to override the influence of the sex-chromosomes and tip the balance of the whole complex in the other direction. This would result in an organism with the sex-chromosomal constitution of one sex developing into an individual of the other sex. Such cases of sex-reversal have been observed, but they do not affect the conception of the sex-chromosome theory. The sex-ratio of the offspring of such sex-reversed individuals can only be explained on the basis of the sex-chromosome theory, and provides, in fact, some of the most valuable evidence in favour of it.

The remarkable phenomena of tightened linkage and of heteropycnosis of the sex-chromosomes in the heterogametic sex possibly provide, as Haldane [100] and Huxley [120] have suggested, alternative methods of preventing crossing over of genes between the X and Y chromosomes, which would inevitably upset the chromosomal determination of sex and probably would result in the production of intersexes.

Many problems connected with the sex chromosomes remain to be solved. Perhaps the most important of these concerns the origin of male and female heterogamety. It is at present a mystery why in some forms the male is heterogametic, while in others perhaps closely related, the male is homogametic. Apparently female heterogamety has arisen independently in several different groups of animals, but the manner in which it has done so can only be guessed.

The study of the sex-ratio provides a useful means of testing and analysing the sex-chromosome theory. Analyses of the mammalian sex-ratio show that the proportion of chromosomal males at conception is considerably higher than that of females. This male excess may be as much as 50 per cent., and can be accounted for only by assuming selective fertilisation favouring the Y-bearing sperms. This conclusion is not at variance with the sex-chromosome theory, which

postulates the production of X- and Y-bearing sperms in equal numbers, but does not necessarily imply that they effect fertilisation in equal numbers. During intra-uterine life and after birth the incidence of mortality falls heavier on the males than on the females and thus tends to equalise the sex-ratio, which usually approaches unity at birth. Huxley [119] suggests that this differential mortality with heavy male incidence may be due to recessive sex-linked deleterious factors. These factors, situated in the X-chromosome, would be less potent in the females of mammals, owing to the frequent occurrence of their normal allelomorphs in the other X-chromosome. This would not be the case in males in which, owing to the presence of only one X-chromosome, their normal allelomorphs would not be present. Consequently these injurious recessive genes would be capable of expression in the males more often than in the females.

Every organism appears to possess the potentiality of developing into either a male or a female, under the influence of suitable stimuli. This dual potentiality is therefore entirely irrespective of the sex-chromosomes, although they normally direct the development and thus determine the ultimate sex of the organism. Should the other factors in the sex-determining complex override the sex-chromosomes the organism becomes of the opposite sex to that which it normally would have done under the influence of the sex-chromosomes. This phenomenon is sex-reversal. Sex-reversal occurs comparatively often in some forms, while in others it is rare or altogether unknown. Apparently the difficulty of overriding the sex-chromosomes varies in different groups, species, and even races. Sex, as determined by the sex-chromosomes, is more stable in some than in others.

Sex-reversal may occur at any time during the life cycle. When it takes place before the gonads are differentiated development proceeds normally and there is no visible abnormality. The fact that it has occurred can then be told only by the effects on the sex-ratio of the offspring of such individuals. Thus, if a sex-reversed XX individual is mated with a normal XX individual all the offspring will be XX. If an

XY sex-reversal is mated with an XY normal the offspring will be in the proportion of 1XX : 2XY : 1YY (probably non-viable). The sex-ratio of the offspring thus reveals the occurrence of sex-reversal in the parent, provided that it does not occur in the F1 generation also. Sex-reversal may also take place at any time after the differentiation of the gonads. It results then in visible reorganisation of the gonads, resulting in their transformation into those of the other sex. The accessory organs then atrophy more or less completely and those proper to the other sex develop ; at the same time the secondary sexual characters also change over. This is due to the sex-reversal of the gonads resulting in a change over of the hormones produced by them which control these characters. Sometimes some of the characters associated with sex are not influenced by the reversal. This is the case, for instance, in sex-reversed hens which, even when completely transformed into males, retain the carriage and bodily configuration peculiar to the female. Apparently these characters, like those involving the chitinous parts in insects, cannot be changed once they have been developed.

Sex-reversal has been observed to occur in almost every possible direction. Thus, the ovary in fish, frogs, and birds may transform into a testis. The testes in Amphibians and Lepidosiren have been observed to develop oocytes and, in the case of Champy's [52] newt, it was claimed that they transformed completely into ovaries. Sex-reversal in birds takes place in heterogametic individuals, in some fish at least it occurs in the homogametic sex, while in frogs there is evidence that both XX and XY females may transform into males. Agar's [1] observations on Lepidosiren are particularly interesting, since they show that the spermatocytes, after they have attained the resting stage of the prophase of the heterotypic division, can transform into oocytes.

Little is known about the ultimate cause of sex-reversal either when it occurs naturally or as the result of experimental treatment. The experimental methods employed for producing sex-reversals in adults are chiefly operative and result in damage to the gonads. Such experiments as partial or

complete ovariotomy or ovarian transplantation in fowl frequently result in the development of spermatic tissue. Castration of male toads resulting in the hypertrophy of Bidder's organ to form an ovary, and the development of oocytes in the regenerated or transplanted testes of Amphibians, are other examples. It seems that serious damage to the gonads, either ovaries or testes, may result in sex-reversal in certain forms. It is probable that sex-reversal in birds, when it occurs naturally without experimental treatment, may be due to atrophy or pathological destruction of the ovaries. These facts seem to indicate that in the forms mentioned the gonads of one sex, when present, prevent the development of those of the other sex, and that sex-reversal can occur only when this adverse influence is removed by partial or complete ablation of the existing germinal tissue. The fact that ovarian and testicular tissue can co-exist in many forms, as shown by grafting experiments and the occurrence of hermaphrodites, is hard to co-ordinate with this view. Pézard and his associates [190] succeeded in grafting ovaries and testes into fowl of the opposite sex.

The various experiments on delayed fertilisation, the action of high and low temperatures during development, and the reduction of the water content of the eggs, which result in sex-reversal in some fish and Amphibians are of a different nature and are more difficult to explain. It can only be surmised that these external factors override the genetic factors concerned with sex-determination, but the method by which this is effected is obscure.

The most instructive examples of sex-reversal are those which are known to result from genetic factors. The work of Hertwig and of Witschi provides extensive data of this type of sex-reversal in various races of frogs. Moreover, it has been shown that their results can be readily interpreted on Goldschmidt's hypothesis, which was formulated to explain the occurrence of intersexes in the various races and crosses of the moth, Lymantria. The essential concepts of this hypothesis are the assumptions that genetic factors control the development of the gonads, that these factors have definite

values in pure strains, which vary in different races, and that they become effective at a certain stage of development, thus introducing a time factor in sex-reversal. Witschi [241, 243] was able to attribute arbitrary values to these hereditary factors in the different races of frogs, based on the percentages of sex-reversals occurring in them, as Goldschmidt had done for his races of Lymantria. Applying these formulæ to the various racial crosses he was able to show that the expected ratio of males, sex-reversals, and females among the offspring approximated closely to that actually observed.

The application of an essentially similar conception to explain the development of many hermaphrodites has been dealt with at length. This explanation, which involves the assumption of a delay in the appearance of the stimulus determining the differentiation of spermatic tissue, fits the observed structural details of male toads and of ovotestes of pigs as well as other forms.

True hermaphroditism and sex-reversal differ from each other chiefly in that the condition is permanent in the former while the latter only passes through a transitory hermaphrodite stage. It is not necessary to assume that the ovarian and testicular tissue differentiate simultaneously in hermaphrodites. They may do so in succession, owing to a change over in the stimuli determining the sex of the developing gonads. They would, in this case, only differ from sex-reversals in the persistence, instead of the degeneration, of the tissue differentiated first, whether ovarian or testicular. This appears to be what actually happens in male toads and hermaphrodite pigs. The portion of the embryonic gonads most advanced in development differentiates into ovarian tissue; the male-determining stimulus then appears and transforms the remainder, still in the indifferent condition, into testicular tissue. The hypothesis, therefore, brings these cases of hermaphroditism into line with sex-reversals from the developmental point of view. It does not explain why the ovarian and testicular tissue can exist side by side in hermaphrodites, while sex-reversal is accompanied by the degeneration of the existing gonads. It is interesting in this connection that

gonad grafts can take and grow in hosts of the opposite sex, almost, if not quite, as well as in castrated hosts or those of the same sex (Pézard, Sand, and Caridroit [190]).

The remarkable examples of gynandromorph birds can be classified, in the present state of our knowledge, with other lateral hermaphrodites, so far as the gonads and accessory organs are concerned. Their unique interest depends on the corresponding unilateral arrangement of the secondary sexual characters. This arrangement can only be explained by assuming that the tissues of one side of the body are male, and those of the other side female in sex-chromosomal constitution. It must be assumed also that these purely somatic tissues react differently to the hormones produced by the ovarian and testicular tissue ; the tissues of female chromosomal constitution reacting more readily to the female than to the male hormones and *vice versâ*. This hypothesis leads to the conclusion that the influence of the sex-chromosomes is not merely expressed in the sexual organs but in every tissue and cell of the body.

Tentative and incomplete as these conclusions undoubtedly are, they represent, nevertheless, a great advance on those which could be formulated ten years ago. During that period our knowledge of sex has increased with astounding rapidity. Few fields of biological research in recent years have proved as fruitful, in few is there more urgent need for further research. The problems of sex lie very near the core of biology, and their solution affects not only academic biology but also medicine, sociology, agriculture, and many other aspects of human activity. The results so far achieved are sufficient to indicate many new lines which will inevitably, in the course of time, yield results of great importance. It has been the chief aim of this book to point out some of these. Should it stimulate further research in these directions it will have fulfilled its purpose.

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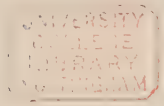

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